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Using the Green Lacewing *Mallada basalis* (Walker) (Neuroptera: Chrysopidae) to Control *Tetranychus kanzawai* Kishida (Acari: Tetranychidae) on Papaya in a Screenhouse

Chien-Chung Chen[1,*], Ling-Lan Cheng[2], Yaw-Jen Dong[3], Chiu-Tung Lu[3], Wen-Jer Wu[4], and John S. Yaninek[5]

Abstract

Chen, C. C., L. L. Cheng, Y. J. Dong, C. T. Lu, W. J. Wu, and J. S. Yaninek. 2014. Using the green lacewing *Mallada basalis* (Walker) (Neuroptera: Chrysopidae) to control *Tetranychus kanzawai* Kishida (Acari: Tetranychidae) on papaya in a screenhouse. J. Taiwan Agric. Res. 63(2):91–104.

Mallada basalis (Walker) is a generalist predator commonly found in agricultural fields in Taiwan, and is commercially available as a biological control product. A number of field investigations have indicated the potential of *M. basalis* as a biological control agent against several arthropod pest species; however, none have been carried out on pests of papayas. Likewise, there is no information on the fate of lacewing populations following their release in the field. This study investigated the feasibility of using *M. basalis* to control the primary acarine pest, *Tetranychus kanzawai* Kishida, on papaya in a screenhouse. The main objectives included determining optimal lacewing release rates, post-release population dynamics, and efficacy in controlling mites on papaya. Lacewings released at a rate of 200 eggs per plant during the summer reduced *T. kanzawai* populations by 95% on average. Mite populations were lower during the winter and required half the summer lacewing release rate to achieve the same level of control. The impact of *M. basalis* on *T. kanzawai* was similar on various life stages of the mite, both sides of the papaya leaves, and throughout the canopy regardless of leaf strata. Lacewing egg hatch success following release in a screenhouse exceeded 70%. However, subsequent larval densities rapidly declined to less than 5 per papaya plant during our weekly censuses. Lacewing populations did not accumulate through successive releases suggesting that conditions in the papaya screenhouse were not favorable for *M. basalis* development or establishment. Although sustained population of *M. basalis* could not be established in the papaya screenhouse, inundative releases of *M. basalis* at a rate of 100 to 200 eggs per plant depending on the season could be applicable as a biological means to control *T. kanzawai* on papaya in screenhouses.

Key words: *Tetranychus kanzawai*, *Mallada basalis*, Biological control, Inundative release, Papaya.

INTRODUCTION

Papaya, *Carica papaya* L., is an important fruit crop in Taiwan with annual production estimated at about 130,000 tons (Anonymous

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2012). The principal cultivated papaya variety in Taiwan is 'Tainung No. 2' (Wang 1991). However, this cultivar is susceptible to the papaya ringspot potyvirus (Lin *et al*. 1989), one of the most destructive diseases of papaya (Purcifull *et al*. 1984). Most papayas in Taiwan are now grown in screenhouses to be protected from aphids, which serve as vectors for papaya ringspot virus. Nevertheless, the unventilated, warm conditions in screenhouses favor outbreaks of acarine pests (Hao *et al*. 1996).

The Kanzawa spider mite, *Tetranychus kanzawai* Kishida, is the major acarine pest of papayas in screenhouses in Taiwan (Ho *et al*. 1997). They feed on cell chloroplasts on the under surface of the leaf, causing the upper surface of the leaf to develop a characteristic whitish or yellowish stippling, which joins and becomes brownish as mite feeding continues (Helle & Sabelis 1985; Yamada & Tsutsumi 1990; Zhang 2003). Heavy damage causes wilting and defoliation, which further reduces plant growth.

Control of *T. kanzawai* on the papaya depends mainly on chemical applications. However, the intensive application of miticides in combination with the short life cycle and high reproductive rates of mites have led to the development of resistance in the Kanzawa spider mite to many registered miticides (Cranham & Helle 1985; Goka 1998; Aiki *et al*. 2005). The number of miticides that can be used is further limited because many miticides produce unacceptable phytotoxicity to papaya (Lo 2002). Consequently, alternative measures are needed to control this papaya pest mite.

With growing concerns about environmental issues, environmentally friendly approaches have been the trend in agricultural pest management. Biological control, which utilizes carefully screened/selected natural enemies to suppress pest populations, is considered an environmentally safe method and a viable alternative to pesticides. The effectiveness of biological control is often greater in glasshouses or screenhouses than in open fields because it is easier to establish sufficient populations of natural enemy through inoculative or inundative release in an enclosed environment.

The green lacewing, *Mallada basalis* (Walker), is common in agricultural fields in Taiwan. The adults feed on nectar and honeydew, but larvae are generalist predators (Wu 1995). This lacewing is mass produced in a cost-effective manner using a microencapsulated artificial diet (Lee 2003), and is commercially available as a biological control product (authorized by Taiwan Agricultural Research Institute). In addition, cold storage techniques have been established for various life stages of this predator, which helps in shipping and scheduling releases (Wu 1992). There have been investigations suggesting its potential as a biological control agent against several species of arthropod pests, including *Phyllocnistis citrella* Stainton, *Aphis* spp., *Nipaecoccus filamentosus* (Cockerell), *Diaphorina citri* Kuwayama, and *Panonychus citri* on citrus; *A. gossypii* Glover on sweet pepper; *T. urticae* Koch and *T. kanzawai* on strawberry; and *P. citri* on Indian jujube (Lo 1997; Lu & Wang 2006). However, no field information is available for this lacewing in controlling mite pests on papayas.

Our previous laboratory studies demonstrated that all larval instars of *M. basalis* were active searchers exhibiting high prey acceptability of *T. kanzawai*, relatively short handling times and voracious consumption of all life stages of the mite (Cheng *et al*. 2009). In choice tests, *M. basalis* did not show an age-class preference for *T. kanzawai* (Cheng *et al*. 2010). A laboratory assessment showed that a predator : prey ratio of 1 : 30 significantly re-

duced *T. kanzawai* densities of all life stages; however, population suppression was better at higher predator-prey ratios (1 : 15 and 1 : 10) (Cheng *et al*. 2012a). A temperature study showed that *M. basalis* was capable of preying on the mites at temperatures as low as 15℃ with prey consumption increasing with temperature up to 30℃ above which rates declined (Cheng *et al*. 2012b).

Although laboratory studies showed *M. basalis* has great potential as a biological control agent against *T. kanzawai*, more field investigations are needed to evaluate its field efficacy before applying this lacewing in papaya biological control programs. This study was therefore conducted in a field setting to investigate the feasibility of using *M. basalis* to control *T. kanzawai* on papaya in a screenhouse. The objectives were to determine optimal lacewing release rates, post-release population dynamics, and efficacy in controlling mites on papaya.

MATERIALS AND METHODS

Experimental fields

The experiment was conducted in four screenhouses that were adjacent to each other at the experimental farm of Taiwan Agricultural Research Institute in Wufeng District of Taichung City, Taiwan. Treatments include a control screenhouse on the far left with lacewing egg release treatments (lowest to highest) to the right of the control. Each screenhouse was 12 m × 27 m, and consisted of 5 beds separated from each other by 0.5 m wide ditches. A total of 40 papaya plants were cultivated in each screenhouse with 8 papaya plants per bed spaced 3 m apart. Irrigation and management were standard for the region except that no insecticide or miticide was applied. The variety 'Tainung No. 2' was planted with seedlings about 25 cm tall on April 20, 2009. The plants were removed on August 5 as the summer investigation finished, and replanted on November 16 for the winter investigation and kept growing until February 24, 2010.

Release of green lacewings

Strips of *M. basalis* eggs obtained from our laboratory colony were used for the release treatments. The eggs were in the late stage of development, consequently, they were black in color with some individuals emerged from hatched eggs. Releases were made on papaya plants starting 3 to 4 weeks after planting when the first signs of *T. kanzawai* were observed on May 13, 2009 in the summer, and December 9, 2009 in the winter investigations, respectively, and were continued once a week for the next 8 weeks. The egg strips were stapled to the upper surface of the leaves in the middle strata of each plant with eggs in contact with the leaf surface between 15:00–16:00 hours. Each egg strip contained 100 eggs. Releases of 100-, 200-, and 300-eggs per papaya plant, plus an untreated check as a control, were implemented separately in individual screenhouses. Released egg strips were brought back to the laboratory after staying on the papaya plants for 3 days, and 10 randomly selected egg strips from each release treatment were examined for egg hatch rates. This evaluation was conducted twice, from May to July, and again from December to February to compare control efficacies between seasons.

Sampling and laboratory examination

Lacewings and mites were sampled from 12 randomly selected papaya plants from each screenhouse. Counts of all active stages of *M. basalis* found per plant stratum/leaf surface were conducted between 14:30 and 17:00 hours on the second, third, and fourth day after and one day prior to lacewing eggs releases.

T. kanzawai densities were estimated from three leaves selected from the upper, middle, and lower third of the foliage of each plant with counts coming from a piece of leaflet about 100 cm^2 in dimension covering the leaf midvein to the leaf margin. Individual leaflet samples were placed in labeled plastic bags and brought back to the laboratory for the mite counts. Mite sampling was conducted in the morning of the weekly lacewing releases, and continued 3–4 more times at the same weekly interval after the last lacewing release. Individual plants were not re-sampled within a 3-wk period, and newly produced leaves were not sampled.

All mite life stages (egg, larva, nymph, and adult) were counted in the laboratory under a microscope and recorded separately for upper versus lower leaf surfaces from upper, middle and lower plant strata. Control efficacy was computed as the number of mites in the untreated check minus the number of mites in the specific release rate treatment divided by the number of mites in the untreated check each week lacewing were released, and then averaged across all weeks of lacewing releases for summer and winter investigation, respectively.

During the investigation periods, temperatures and relative humidity in the screenhouses were monitored using an Onset HOBO data logger. Papaya plant heights were recorded once a week.

Data analyses

All data except of those for the weekly population dynamics of lacewings and mites counts were averaged across sampling dates during the summer and the winter investigation, respectively. Normally distributed data were analyzed using analysis of variance (ANOVA), and non-normally distributed data were analyzed with the Kruskal-Wallis test. Means were separated for significance using the Fisher's protected LSD procedure. For pair-wise comparisons, a *t*-test was used to analyze normally distributed data, and a Mann-Whitney test was used to analyze non-normally distributed data. The significance level was set at $P < 0.05$. All analyses were conducted using STATGRAPHICS Centurion XV, software, 2005 (Statpoint, Inc, Herndon, VA).

RESULTS

Egg hatch rates of *M. basalis* after released on papaya plants during the summer season measured 72.13% ± 1.98% on average (with a range of 58.89% ± 2.87% to 87.27% ± 2.62%), and during the winter season measured 73.11% ± 2.50% on average (with a range of 56.89% ± 4.11% to 86.43% ± 1.39%). There were no significant differences in hatch rate among the various release rates (Summer: F = 0.1, P = 0.9043; Winter: F = 0.06, P = 0.9462) (Fig. 1). The number of lacewing larvae observed on papaya plants declined by more than 85% within 3 days after egg release (Fig. 2). During the weekly investigations, less than 5 lacewing larvae per plant were found even

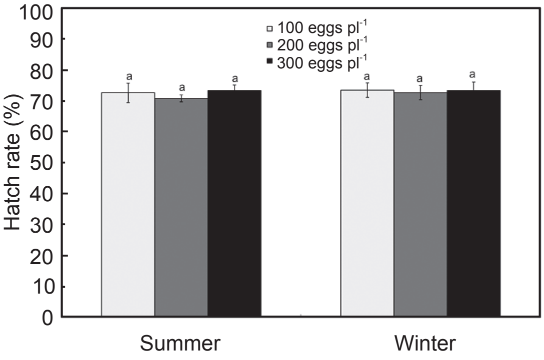

Fig. 1. Hatch rates of *Mallada basalis* eggs released on papaya plants at three rates in different seasons. Hatch rates were measured three days after egg release. Means with the same letter for each season are not significantly different at P < 0.05 (F-test; STATGRAPHICS Centurion XV, 2005). Error bars represent ± SEM.

with the highest release rate of 300 eggs per plant (Fig. 3). Lacewing post-release populations were lower during the winter than the summer, and the populations did not accumulate and increase through sequential releases in either season. Lacewing distributions, according to the observations, were not different either across plant strata or between upper versus lower surfaces of leaves. Even though some pupae were found during our census in the summer, no adults or second-generation eggs were observed.

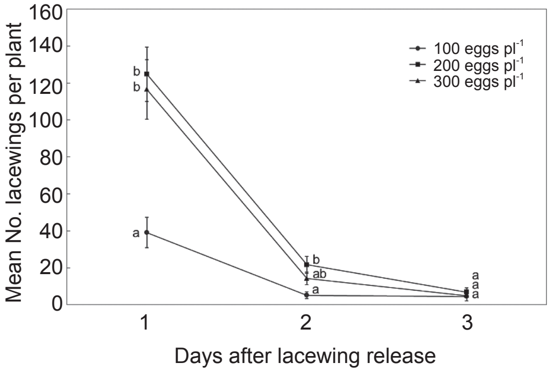

Fig. 2. Number of *Mallada basalis* observed on papaya plants within 3 days of release. Data were collected from the winter investigation, and averaged across 7 sequential release dates. Means with different letters on each date are significantly different at $P < 0.05$ (F-test and Fisher's protected LSD procedures; STATGRAPHICS Centurion XV, 2005). Error bars represent ± SEM.

T. kanzawai populations in the 100, 200, and 300 lacewing eggs per plant treatments were 65.7, 95.4, and 95.5% lower, respectively, compared to the control after 8 weeks of *M. basalis* egg releases in the summer (Fig. 4). *T. kanzawai* populations during the winter were near zero compared to the control after seven sequential releases, regardless of lacewing egg

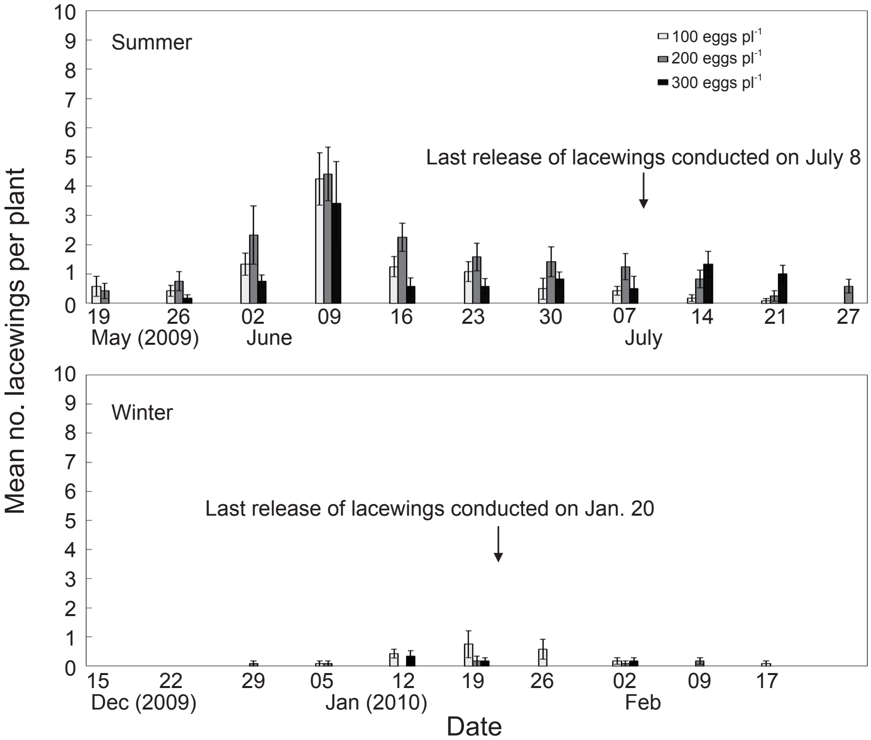

Fig. 3. Mean number of *Mallada basalis* observed per papaya plant for three different lacewing egg release rates during summer and winter. Error bars represent ± SEM. Investigations were conducted once a week on one day before each sequential release, and continued for 3–4 more weeks after the last release.

release rates (Fig. 4). After lacewing releases were completed, the mite populations increased in the screenhouses that received 200 and 300 egg release treatments, but decreased in the control and 100 egg treatments. Most papaya leaves in the control and 100 egg treatments had wilted severely from *T. kanzawai* injury at this time (pers. obs.). This was more prominent during the summer compared to the winter investigation.

T. kanzawai eggs were the most frequently observed life stage, followed by nymphs, then adults (Table 1). In the summer, most mites were distributed on the lower foliage of the papaya plant, but dispersed to the middle, then the upper strata as mite populations increased and the plants added leaves (Table 2). During the winter, similar or more mites were found on the middle plant stratum (Table 2). Almost all mites were found on the undersurface of papaya leaves (Table 3). These distribution patterns were not significantly different across the untreated check and the various rates of lacewing release treatments ($P > 0.05$, ANOVA) (Tables 1–3).

Lacewing control efficacy during the summer were not different in the treatments that received releases of 200 and 300 eggs per plant, but was significantly lower in the treatment that received releases of 100 eggs per

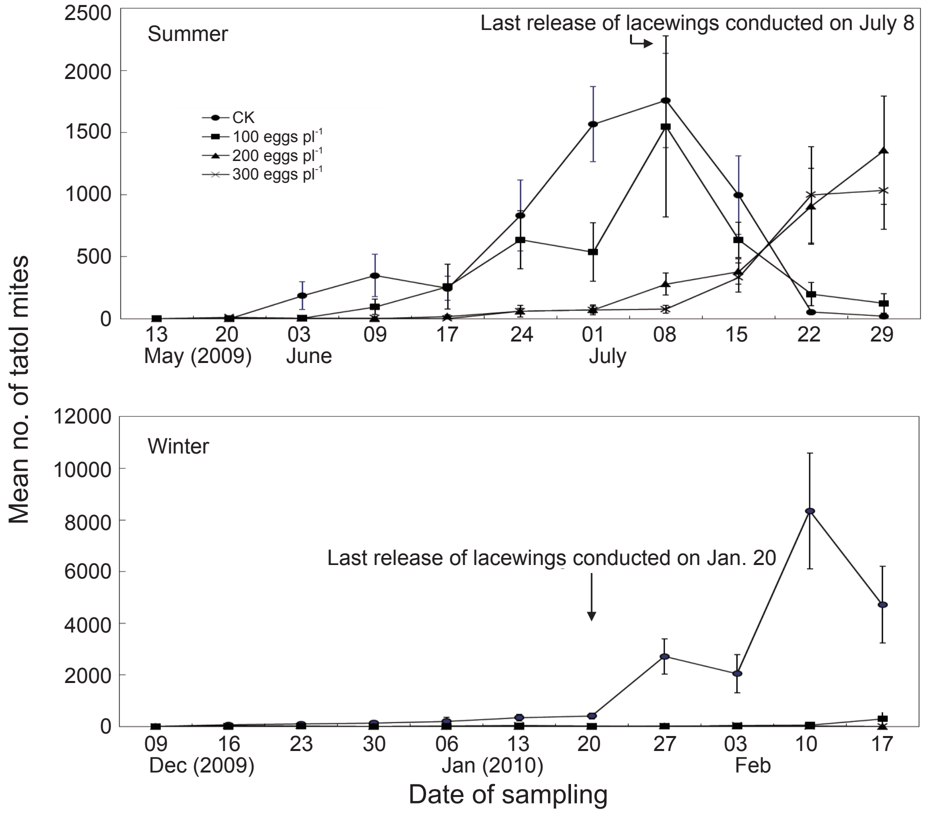

Fig. 4. Number of *Tetranychus kanzawai* observed from three leaf blade segments (approximately 100 cm^2 per leaf segment) collected respectively from the lower, middle, and upper third of papaya plants treated with different release rates of *Mallada basalis*. Error bars represent ± SEM. Sampling was conducted the same day prior to the weekly lacewing releases, and continued for 3–4 more weeks after the last release. Based on observations, most of the papaya leaves in the control and 100 egg treatments wilted severely from *T. kanzawai* injury after July 1 and February 10.

Table 1. Age class distribution of *Tetranychus kanzawai* on papaya treated with various release rates of *Mallada basalis*.

Investigation period	Lacewings released per plant	% Mite proportion (Mean ± SE)[z]			
		Egg	Larva	Nymph	Adult
Summer	100 eggs	56.3 ± 6.8 a	7.2 ± 3.4 a	23.7 ± 4.2 a	12.9 ± 4.2 a
	200 eggs	63.6 ± 2.6 a	4.6 ± 1.5 a	20.1 ± 2.5 a	11.7 ± 2.4 a
	300 eggs	63.4 ± 5.3 a	4.8 ± 2.0 a	22.3 ± 6.3 a	9.6 ± 3.5 a
	Control	60.8 ± 3.9 a	7.0 ± 1.8 a	23.5 ± 3.9 a	8.2 ± 1.1 a
Winter	100 eggs	62.4 ± 5.5 a	8.1 ± 3.0 a	18.7 ± 3.0 a	10.7 ± 2.4 a
	200 eggs	61.3 ± 5.4 a	7.2 ± 2.6 a	18.8 ± 5.0 a	12.5 ± 4.7 a
	300 eggs	62.1 ± 6.2 a	11.5 ± 2.4 a	17.2 ± 4.5 a	9.2 ± 2.7 a
	Control	72.5 ± 3.3 a	8.7 ± 2.1 a	11.5 ± 1.9 a	7.4 ± 1.1 a

[z] Means within the same column for a given season followed by the same letter are not significantly different at $P < 0.05$ (F-test; STATGRAPHICS Centurion XV 2005).

Table 2. Distribution of *Tetranychus kanzawai* on the lower, middle and upper third of the papaya leaf strata treated with various release rates of *Mallada basalis*.

Investigation period	Lacewings released per plant	% Mite proportion (Mean ± SE)[z]		
		Lower	Middle	Upper
Summer	100 eggs	74.3 ± 6.6 a	21.3 ± 5.4 a	4.4 ± 1.8 a
	200 eggs	74.3 ± 5.1 a	22.3 ± 5.4 a	2.2 ± 1.0 a
	300 eggs	71.7 ± 6.0 a	24.5 ± 6.4 a	3.9 ± 2.6 a
	Control	68.7 ± 4.6 a	27.5 ± 4.2 a	5.7 ± 2.2 a
Winter	100 eggs	46.8 ± 10.3 a	48.4 ± 10.2 a	4.8 ± 2.0 a
	200 eggs	34.6 ± 8.9 a	56.8 ± 8.5 a	8.7 ± 3.1 a
	300 eggs	33.4 ± 8.5 a	62.9 ± 8.3 a	3.7 ± 2.5 a
	Control	26.6 ± 7.1 a	61.8 ± 7.1 a	11.6 ± 2.1 a

[z] Means within the same column for a given season followed by the same letter are not significantly different at $P < 0.05$ (F-test; STATGRAPHICS Centurion XV 2005).

Table 3. Distribution of *Tetranychus kanzawai* on the upper and lower surfaces of papaya leaves treated with various release rates of *Mallada basalis*.

Investigation period	Lacewings released per plant	% Mite proportion (Mean ± SE)[z]	
		Uppersurface	Undersurface
Summer	100 eggs	2.2 ± 1.6 a	97.8 ± 1.6 a
	200 eggs	0.3 ± 0.2 a	99.7 ± 0.2 a
	300 eggs	1.3 ± 1.3 a	98.7 ± 1.3 a
	Control	1.1 ± 0.5 a	99.0 ± 0.5 a
Winter	100 eggs	2.9 ± 2.9 a	97.1 ± 2.9 a
	200 eggs	0.0 ± 2.0 a	100.0 ± 0.0 a
	300 eggs	0.0 ± 0.0 a	100.0 ± 0.0 a
	Control	0.5 ± 0.3 a	99.5 ± 0.3 a

[z] Means within the same column for a given season followed by the same letter are not significantly different at $P < 0.05$ (F-test; STATGRAPHICS Centurion XV 2005).

plant (Table 4). However during the winter, similar high control effectiveness (> 95%) was achieved for all three release rates (Table 4). The impact of *M. basalis* on *T. kanzawai* was similar on each life stage of the mite ($P > 0.05$, ANOVA) (Tables 1 and 4), as well as both sides of the papaya leaves ($P > 0.05$, *t*-test) (Tables 3 and 6), and throughout the canopy regardless of leaf strata (Tables 2 and 5) ($P > 0.05$, ANOVA).

During the summer, papaya heights ranged between 30–200 cm and temperatures averaged 30.5℃ (ranged 19.4–45.7℃); during the winter, papaya heights ranged between 30–80 cm and temperatures averaged 18.5℃ (ranged 5.1–40.0℃).

DISCUSSION

There have been investigations demonstrating the control effectiveness of *M. basalis* against several species of arthropod pests, including *Phyllocnistis citrella*, *Aphis* spp., *Nipaecoccus filamentosus*, *Diaphorina citri*, *T. kanzawai*, *T. urticae*, and *P. citri* in the field (Wu 1992; Chang & Huang 1995; Lo 1997;

Table 4. Mean control efficacy (± SE) of *Mallada basalis* on various mite life stages of *Tetranychus kanzawai* on papaya treated with various release rates of *M. basalis*.

Lacewings released per plant	Control efficacy (%)[z]				
	Egg	Larva	Nymph	Adult	Total mites
Summer					
100 eggs	32.0 ± 18.6 a	58.0 ± 14.5 a	58.4 ± 15.1 a	34.1 ± 15.8 a	40.5 ± 17.3 a
200 eggs	93.6 ± 2.4 a	97.5 ± 1.2 a	96.8 ± 1.2 a	96.2 ± 1.7 a	95.2 ± 1.7 b
300 eggs	95.8 ± 2.1 a	94.9 ± 3.8 a	97.2 ± 0.9 a	97.3 ± 0.9 a	96.5 ± 1.5 b
Winter					
100 eggs	94.7 ± 2.2 a	97.0 ± 1.3 a	90.4 ± 4.2 a	96.3 ± 1.4 a	95.0 ± 1.8 a
200 eggs	99.2 ± 0.3 a	98.0 ± 1.6 a	90.6 ± 8.4 a	98.1 ± 1.4 a	98.7 ± 0.6 a
300 eggs	96.8 ± 2.4 a	94.8 ± 2.6 a	93.8 ± 3.9 a	98.4 ± 1.3 a	96.5 ± 2.4 a

[z] Means within the same row for the various mite life stages followed by the same letter are not significantly different at $P < 0.05$ (*F*-test; STATGRAPHICS Centurion XV, 2005), and mean total mites within the same column for a given season followed by different letters are significantly different at $P < 0.05$ (*F*-test and Fisher's protected LSD procedures; STATGRAPHICS Centurion XV 2005).

Table 5. Mean control efficacy (± SE) of *Mallada basalis* on *Tetranychus kanzawai* on the lower, middle and upper third of the papaya leaf strata treated with various release rates of *M. basalis*.

Lacewings released per plant	Control efficacy (%)[z]		
	Lower	Middle	Upper
Summer			
100 eggs	48.7 ± 15.2 a	29.8 ± 14.4 a	70.3 ± 23.7 a
200 eggs	93.5 ± 2.3 a	98.3 ± 1.2 a	93.8 ± 4.4 a
300 eggs	94.9 ± 2.7 a	97.2 ± 1.2 a	99.9 ± 0.1 a
Winter			
100 eggs	95.2 ± 2.5 a	95.1 ± 1.7 a	92.3 ± 7.0 a
200 eggs	92.8 ± 6.1 a	99.6 ± 0.2 a	99.9 ± 0.1 a
300 eggs	91.4 ± 5.0 a	97.7 ± 1.3 a	100.0 ± 0.0 a

[z] Means within the same row followed by the same letter are not significantly different at $P < 0.05$ (*F*-test; STATGRAPHICS Centurion XV 2005).

Table 6. Mean control efficacy (± SE) of *Mallada basalis* on *Tetranychus kanzawai* on upper and lower surfaces of papaya leaves treated with various release rates of *M. basalis*.

Lacewings released per plant	Control efficacy (%)[z]	
	Uppersurface	Undersurface
Summer		
100 eggs	35.9 ± 22.1	39.9 ± 17.7
200 eggs	96.4 ± 2.2	95.1 ± 1.7
300 eggs	97.2 ± 2.8	95.6 ± 1.4
Winter		
100 eggs	100.0 ± 0.0	94.9 ± 1.8
200 eggs	100.0 ± 0.0	98.7 ± 0.6
300 eggs	100.0 ± 0.0	96.5 ± 2.4

[z] Means within the same row are not significantly different at $P < 0.05$ (*t*-test; STATGRAPHICS Centurion XV 2005).

Hao 2002; Lu & Wang 2005). However, no information is available on detailed tracking of the lacewing population after field release. The current study found that *M. basalis* egg hatch following release in a screenhouse was consistently high in both summer and winter seasons. Egg predation by ants (eggs were cut from their stalks and moved to ant nests) was observed to be the main factor affecting lacewing egg hatch; cannibalism by conspecifics played only a limited role on the hatch rate, which might explain the similarity in hatch rate among the various lacewing release rates.

M. basalis larvae from recently hatched eggs were observed actively moving throughout the papaya foliage and on leaf surfaces, petioles, stems, surrounding weeds, as well as the ground. There was no evidence that *M. basalis* preferred to inhabit or forage in specific locations on or around the plant. Larval densities declined rapidly within 3 days of egg release. Only a few lacewing larvae were found during the weekly census even for the highest release rate. Consequently, lacewing populations did not accumulate through successive releases suggesting that the mass-produced *M. basalis* were unable to grow and reproduce in the papaya screenhouses. It has long been thought that one of the reasons biological control agents fail to establish in the field is because of the impact of inter-guild predation. Rosenheim *et al*. (1999) documented that five species of generalist predators in the order Hemiptera were the potential source of *Chrysoperla carnea* mortality in the cotton field. The relatively high rates of successful hatch observed in our study suggested that it was the *M. basalis* young larvae that were vulnerable to significant predation mortality. In our papaya screenhouses, ants were the only specific predators of *M. basalis* we observed. We often observed ants moving lacewing prey and lacewings attacking their conspecifics. These two factors seemed to be the major source of predation acting on the young lacewing larvae.

Climatic conditions are also important determinant of organism survival. Chang (2000) indicated that the effective temperature for *M. basalis* development ranged from 15–43℃. The temperatures monitored in the screenhouses during our experimental periods were mostly within the upper limit during the summer, but more often dropped below the lower limit during the winter. Low temperatures during the winter might be a cause of *M. basalis* mortality, but not in the summer.

Green lacewings are generalist predators that feed on a broad array of small arthropods

(Canard & Principi 1984). Under conditions of prey scarcity, they can exploit plant-based resources, e.g., extrafloral nectar (Limburg & Rosenheim 2001). Limburg & Rosenheim (2001) indicated that extrafloral nectar did promote substantial longevity of first-instar lacewing larvae, although extrafloral nectar contributed only slightly to neonate lacewing growth and did not support lacewing development. We noticed fewer extrafloral nectaries along the leaf veins and stems of papaya plants that received lacewing release treatments compared to the untreated plants. We speculate that lacewing larvae might feed on nectaries in the screenhouse, based on direct observations under a microscope in the laboratory. Besides extrafloral nectaries, several species of *M. basalis*' natural prey occurred in our papaya screenhouses including whiteflies, larvae of tobacco cutworm, and rusty plum aphids (inhabiting goosegrass). Food limiting should not explain the sharp population drop of the first instars of *M. basalis* after successful hatch. This presumption was supported by the rapid population decline of the first instar lacewings in the 100-egg treatment screenhouse when the *T. kanzawai* population densities were high.

Generalist predator may not always respond to prey-specific cues. We observed *M. basalis* in the laboratory that searching for mite prey in a random pattern, and requiring potential prey to be within a range of a few millimeters before they are recognized by physical contact. We often found *M. basalis* in the screenhouses, not only foraging in the plant canopy, but also moving along plant stems to the ground or the surrounding weeds. Papaya has a more open canopy compared to other plants, which may be a reason lacewing larvae have a greater tendency for these movements. Moving off the plant could subject the lacewings to greater predation pressure (especially ant predation), and harsher environments including the extreme surface temperatures found on soils in the sun light. We found that *M. basalis* stayed and grew better on bitter gourd with aphid prey than on papaya with mites (pers. obs.). Whether the differences in the lacewing aggregation and development are a result of differences in attractiveness between plant species or between prey species requires further investigation.

M. basalis did not grow well in the screenhouse in this study, but did generate good control against *T. kanzawai*. Cheng *et al*. (2009) demonstrated that *M. basalis* was a voracious predator with the first-instar larva consuming an average of 20 *T. kanzawai* eggs in 2 hours. The lacewings might have attacked most of the mites in the experimental screenhouses before their population declined to low levels, or they might move out of our sight, but come back to the plant to forage at some other time.

There have been several field investigations demonstrating the effectiveness of *M. basalis* in controlling acarine pests. Chang & Huang (1995) reported that *M. basalis* released at the rate of 5 first instar larvae per plant once every 3 weeks for a total of three releases reduced *T. kanzawai* populations on strawberry by 60%. Hao (2002) indicated that *M. basalis* released at 200 eggs per plant once every 7–10 days (9 successive releases) reduced *P. citri* populations on Indian jujube in net houses by 90%. Wu (1992) demonstrated that *M. basalis* released at a rate of 1,000 eggs per tree effectively reduced *P. citri* on citrus. The current study demonstrated that *M. basalis* released at a rate of 200 eggs per plant once every week reduced *T. kanzawai* populations during the summer by 95%. Mite populations were lower during the winter and could be controlled by the lower release rate of 100 eggs per plant to achieve the same level of control observed dur-

ing the summer. Cheng *et al*. (2012b) reported that consumption of *T. kanzawai* by 2nd instar *M. basalis* increased between 15 and 30℃ , and then declined between 30 and 35℃. Although lacewing predation was lower during the winter, *T. kanzawai* also has a lower population growth at lower temperatures. On the other hand, *T. kanzawai* population growth rates during the summer are optimal at 34℃ (Chang 2000), which is beyond the optimum temperature range for *M. basalis* predation. These might explain why *M. basalis* was better at controlling these mites in the winter than in the summer. However, the period of effective lacewing control after termination of releases could not be measured accurately in this study because the mites began migrating from the untreated screenhouse to the lacewing-treated screenhouses, perhaps because of food deprivation, thus interfering with the regular paces of mite population increases.

Mite eggs were the most frequently observed age class of *T. kanzawai* followed by nymphs, then adults in papaya screenhouses, which was consistent with prior knowledge (Zhang 2003). Mites were most abundant in the lower and middle vertical strata of the plant canopy and least abundant on the young leaves in the upper canopy. Furthermore, mites were found predominantly on the lower surface of leaves. When populations reached relatively high densities in the lower plant canopy, mites tended to move from the lower to the upper plant strata. Differences in predator-prey relative mobility and spatial distribution, and prey nutritional value may result in predators preferring or only being capable of attacking certain life stages of prey, or prey at specific localities. This often affects the effectiveness of biological control agents against their target pest. Our results showed that the age class distribution of *T. kanzawai* and their population distribution over different papaya plant strata and leaf surfaces for different lacewing release rate treatments and the untreated check were not significantly different. It was also shown that control efficacies were similar for various mite life stages and on papaya leaf/canopy localities. Besides, *M. basalis* were active at the various papaya localities (papaya heights within 2 m) with similar frequency. These suggest that *M. basalis* contains *T. kanzawai* of all life stages across the entire papaya plant. It corresponds to our previous findings from a laboratory study that *M. basalis* does not discriminate among various life stages of *T. kanzawai*, and suppresses mites of all life stages and on both sides of papaya leaves (Cheng *et al*. 2012a, 2012b). These are important attributes for *M. basalis* to be an effective biological control predator for papaya mite pest management.

The environmental conditions of screenhouses are easier to manipulate than those in the open field, thus should increase the success of biological control applications. There has been research suggesting that the use of lacewings is best suited to glasshouses or other enclosed systems (Daane *et al*. 1998). The current study indicated that environmental conditions in the papaya screenhouses are still not favorable for *M. basalis* development and population establishment. Nevertheless, the lacewing at the release rate of 200 eggs per plant did provide good control against papaya pest mites, and release rates could be further reduced to 100 eggs per plant for the winter application. *M. basalis* can be mass-produced on artificial diet in a cost-effective manner. With the current techniques, production of a *M. basalis* adult has been estimated being about 0.028 USD including diets, labor, utility, and facility, and one female can produce an average of 736.3 eggs per lifetime. Therefore, each release of *M. basalis* for controlling *T. kanzawai* on papaya

costs about 40 USD ha^{-1}. Although our study suggested that control of *T. kanzawai* on papaya would not be achieved by inoculative releases of *M. basalis*, inundative releases as a biological pesticide should be viable.

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應用基徵草蛉 (Neuroptera: Chrysopidae) 防治網室木瓜上神澤氏葉蟎 (Acari: Tetranychidae) 之研究

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摘要

陳健忠、鄭玲蘭、董耀仁、盧秋通、吳文哲、J. S. Yaninek。2014。應用基徵草蛉 (Neuroptera: Chrysopidae) 防治網室木瓜上神澤氏葉蟎 (Acari: Tetranychidae) 之研究。台灣農業研究 63(2):91–104。

基徵草蛉 *Mallada basalis* (Walker) 屬非專一性的捕食性昆蟲，為台灣農作區常見的天敵種類，可利用人工飼料加以大量繁殖，具經濟效益並已商品化。往昔研究顯示基徵草蛉可應用於田間防治多種作物的重要害蟲及害蟎，但於網室木瓜尚無正式報告。此外，對於草蛉釋放後，其族群在田間變動的情形也少有探討。本研究乃探討網室中釋放基徵草蛉防治木瓜害蟎之可行性，主要項目包括草蛉的有效釋放率、釋放後草蛉在田間的族群變動情形、以及其對木瓜上神澤氏葉蟎 *Tetranychus kanzawai* Kishida 的防治效能。結果顯示每株木瓜釋放 200 粒草蛉卵，夏天每次釋放後的平均防治率可達 95% 以上，冬天由於葉蟎族群密度較低，草蛉釋放率可降至每株木瓜 100 粒卵，即達相同的防治效果。此外，基徵草蛉對木瓜植株上不同生活期、不同葉層或不同葉表的葉蟎，防治效果皆無顯著差異。草蛉卵釋放於木瓜網室後的孵化率達 70% 以上，然而稚齡幼蟲孵化後，其族群密度即迅速降低。草蛉在木瓜網室中無法經由連續釋放累積其族群數量，顯示木瓜網室無法提供草蛉生長發育所需的適當環境。雖然基徵草蛉無法在木瓜網室中建立族群，本研究顯示以淹沒式釋放方式 (inundative release)，在每株木瓜上視季節釋放 100–200 粒基徵草蛉卵，可有效防治木瓜上之神澤氏葉蟎，具應用潛力。

關鍵詞：神澤氏葉蟎、基徵草蛉、生物防治、淹沒式釋放、木瓜。

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A List of Chionaspinae (Hemiptera: Sternorrhyncha: Coccoidea: Diaspididae) Deposited in the Insect Collection of Taiwan Agricultural Research Institute

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Abstract

Chen, S. P., J. Y. Wong, and W. J. Wu. 2014. A list of Chionaspinae (Hemiptera: Sternorrhyncha: Coccoidea: Diaspididae) deposited in the Insect Collection of Taiwan Agricultural Research Institute. J. Taiwan Agric. Res. 63(2):105–121.

A list of 39 species in 9 genera of Chionaspinae (Hemiptera: Sternorrhyncha: Coccoidea: Diaspididae) with 246 slide mounted specimens deposited at Insect Collection of the Taiwan Agricultural Research Institute (TARI) is provided. The specimens were mainly collected during Japanese Colonial Period. The synonyms, host plants, distribution and photographs of slide-mounted specimen are also provided for each species.

Key words: Chionaspinae (Diaspididae), Slide-mounted specimens, Taiwan Agricultural Research Institute (TARI).

INTRODUCTION

Scale insects (Hemiptera: Sternorrhyncha: Coccoidea) of Taiwan have been extensively studied by serveral western and Japanese entomologists such as Green, Ferris and Takahashi since 1923 (Tao 1978). Most of the slide-mounted specimens of Chionaspinae (Hemiptera: Sternorrhyncha: Coccoidea: Diaspididae) collected by Takahashi were deposited at the Insect Collection, Division of Applied Zoology, Taiwan Agricultural Research Institute (TARI). Among them, a great number of slide-mounted specimens were types designated by Takahashi. However, a considerable number of species names used by Takahashi have been changed or synonymized with other names. In this study, taxonomic status of all slide-mounted specimens were examined and revised based on the current studies.

MATERIALS AND METHODS

A total of 246 slide-mounted specimens were examined by using a Leita DMRB light microscope (Leica, Milton Keynes, U.K.) in this study. Distribution data of each species were compiled based on the information provided on the slide-mounted specimens deposited at the TARI Insect Collection. Photographs of slide-mounted specimens (adult female) were taken by using Leita DMRB light microscope, Automatic Piezo stepping motor (Leica Piezo Z-drive, Switzerland) and Leica DFC 420 Digital

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Microscope Cameras (Leica, Switzerland) for each species. The old name and current name of collection locality records are included in the paper based on guidelines developed by Chu & Yamanaka (1973, 1974, 1975) and the Taiwan Geographic Name Information Systems (http://placesearch.moi.gov.tw/search/index.php).

RESULTS

Among the identified 39 species (9 genera) of Chionaspinae in TARI, there were 22 species described by Takahashi, 5 species described by Kuwana, and 5 species described by Green. In the study, there are 10 species not collected in Taiwan, including *Chionaspis lithocarpicola* Takahashi, *Chionaspis salicis* (Linnaeus), *Kuwanaspis linearis* (Green), *Nikkoaspis sasae* (Takahashi), *Nikkoaspis shiranensis* Kuwana, *Pinnaspis javanensis* (Kuwana), *Pinnaspis siamensis* Takahashi, *Unaspis acuminata* (Green), *Unaspis citri* (Comstock), and *Unaspis euconymi* (Comstock). The following list provides comprehensive information on the nomenclature, synonymies, host plants, and distribution of each species. Table 1 is a check list of the old name and current name of collection locality records included in the paper (Chu & Yamanaka 1973, 1974, 1975).

***Chionaspis cinnamomicola* (Takahashi 1935)** (Fig. 1A) (Takahashi 1935a; Takagi 1970; Tao 1989; Ben-Dov & Miller 2013)

Original spelling on label: *Diaspis machilicola var. cinnamomicola* Takahashi, 1935. (8 slides)

Distribution: Taiwan (Giran: Kussha; Takao: Chushinron near Rokki).

Host plant: C*innamomum erythrophloia* (Lauraceae).

Syn.: *Diaspis machilicola cinnamomicola* Takahashi, 1935; *Diaspis cinnamomicola* (Takahashi 1935).

Note: Syntypes, female [six slides collected from Taiwan: Taihoku, Kussha near Shikikun, on *Cinnamomum erythrophloia*, 14-VIII-1934, by R. Takahashi (#26-3-3-1–26-3-4-1)].

***Chionaspis lithocarpicola* Takahashi, 1942** (Fig. 1B) (Takahashi 1942b; Ben-Dov & Miller 2013)

Original spelling on label: *Chionaspis lithocarpicola* Takahashi, 1942 (1 slide)

Distribution: Thailand (Mt. Sutep).

Host plant: *Lithocarpus* sp. (Fagaceae).

Note: Syntypes, female [collected from Thailand: Mt Sutep, on *Lithocarpus* sp., 12-IV-1940, by R. Takahashi (#23-4-4-1)].

***Chionaspis machilicola* (Takahashi 1935)** (Fig. 1C) (Takahashi 1935a; Takagi 1970; Tao 1989; Ben-Dov & Miller 2013)

Original spelling on label: *Diaspis machilicola* Takahashi, 1935 (4 slides)

Distribution: Taiwan (Taito: Chipponsan).

Host plant: *Machilus* sp. (Lauraceae).

Syn.: *Diaspis machilicola* Takahashi, 1935.

Note: Syntypes, female [collected from Taiwan: Taito, Chipponsan, on *Machilus* sp., 23-III-1934, by R. Takahashi (#26-3-2-1–4)].

***Chionaspis rotunda* (Takahashi 1935)** (Fig. 1D) (Takahashi 1935a; Takagi 1970; Tao 1989; Ben-Dov & Miller 2013)

Original spelling on label: *Phenacaspis rotunda* Takahashi. (1 slide)

Distribution: Taiwan (Heito: Mutosan).

Host plant: *Quercus pseudomyrsinifolia* (Fagaceae).

Syn.: *Phenacaspis rotunda* Takahashi, 1935; *Chionaspis rotunda* (Takahashi 1935).

Note: Holotype, female [collected from Taiwan: Takao, Heito-Gun, Mutosan, on *Quercus pseudomyrsinifolia*, 23-III-1934, by R, Takahashi (#39-5-3-1)].

Table 1. A check list of the old name, current name and Chinese name of collection localities included in the paper.

Old name	Current name	Chinese name
Botel Tobago	Taitung: Lanyu	台東：蘭嶼
Chikushiko	Taipei: Zhuzihu	台北：竹子湖
Chippon	Taitung: Zhiben (Chihpen)	台東：知本
Chipponsan	Taitung: Zhiben Mountain	台東：知本山
Churei	Yilan: Chungling	宜蘭：中嶺
Chushinron near Rokki	Kaohsiung: Paoshan near Liouguei	高雄：寶山 近 六龜
Daichikko	Taitung: Dazhu	台東：大竹
Garambi, Garanbi	Pingtung: Eluanbi	屏東：鵝鑾鼻
Habon	Nantou: Holuan	南投：合巒
Heito	Pingtung	屏東
Hichiseizan, Hichiseisan	Taipei: Qixing Mountain (Chihsingshan)	台北：七星山
Hinode	Kaohsiung: Rizhong	高雄：旗山：日中
Hokuto	Taipei: Beitou	台北：北投
Hori, Horin	Nantou: Puli	南投：埔里
Hsintien	New Taipei City: Xindian	新北市：新店
Itabashi	New Taipei City: Banqiao	新北市：板橋
Kaede	Taitung: Fenglin Bridge	台東：海端：楓林橋
Kaho	Taoyuan: Hsiapu	桃園：下埔
Kakayo, Kakayom	Taitung: Jiadai (Chiachiadai)	台東：嘉代 (嘉嘉代)
Kanaron, Kanoron	Taitung: Jinlun (Chinlun)	台東：金崙
Kanshirei	Tainan: Guanziling (Kuantyling)	台南：關子嶺
Karenko	Hualien	花蓮
Kontei	Pingtung: Kending	屏東：墾丁
Koshun	Pingtung: Hengchun	屏東：恆春
Kotosho	Taitung: Lanyu	台東：蘭嶼
Kuraru	Pingtung: Sheding	屏東：社頂
Kussha	Yilan: Kuochia	宜蘭：庫霞
Maruyama	Taipei: Yuanshan	台北：圓山
Murorafu, Muroruafu, Mururoafu	Yilan: Jialuo Mountain (Chialoshan)	宜蘭：加羅山
Mutosan	Pingtung: Wutoushan	屏東：霧頭山
Oiwake	Taitung: Zhuifen (Chuifen)	台東：追分
Piyahau	Yilan: Bihou (Pihau)	宜蘭：碧候
Raisha	Pingtung: Laiyi	屏東：來義
Riran	Taitung: Lilong	台東：關山：里龍
Sankyo near Taihoku	New Taipei City: Sansia near Taipei	新北市：三峽 近 台北
Sekinon	New Taipei City: Shimen	新北市：石門
Sekitei	New Taipei City: Shiding (Shihding)	新北市：石碇
Shinten	New Taipei City: Xindian (Sindian)	新北市：新店
Shin-Taiheizan	Yilan: Taiping Mountain (Taipingshan)	宜蘭：太平山
Shirin	Taipei: Shilin	台北：士林
Sozan	Taipei: Yangmingshan	台北：陽明山

Table 1. A check list of the old name, current name and Chinese name of collection localities included in the paper. (continued)

Old name	Current name	Chinese name
Suisha	Nantou: Shuishe	南投：魚池：水社
Suo	Yilan: Su’ao	宜蘭：蘇澳
Taichu	Taichung	台中
Taiheizan, Taihezan, Taiheisan	Yilan: Taiping Mountain (Taipingshan)	宜蘭：太平山
Taihoku, Taipei	Taipei	台北
Tainan	Tainan	台南
Takao Takaoshu	Kaohsiung	高雄
Tamsui Tansui	New Taipei City: Danshui (Danshuei)	新北市：淡水

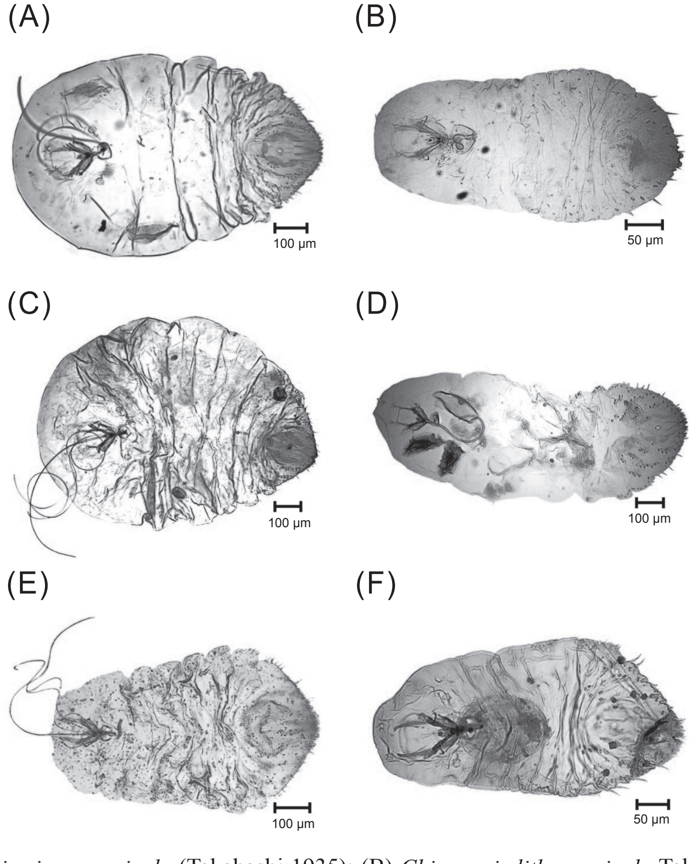

Fig. 1. (A) *Chionaspis cinnamomicola* (Takahashi 1935); (B) *Chionaspis lithocarpicola* Takahashi, 1942; (C) *Chionaspis machilicola* (Takahashi 1935); (D) *Chionaspis rotunda* (Takahashi 1935); (E) *Chionaspis salicis* (Linnaeus 1758); and (F) *Chionaspis sozanica* Takahashi, 1933.

Chionaspis salicis **(Linnaeus 1758)** (Fig. 1E)

(Linnaeus 1758; Chou 1982; Ben-Dov & Miller 2013)

(1) Original spelling on label: *Chionaspis alni* var. *hokkaidensis* Takah. (2 slides)

Distribution: Japan (Hokkaido: Sapporo).

(2) Original spelling on label: *Chionaspis salicis* L. (2 slides)

Distribution: Turkey (Angora).

(3) Original spelling on label: *Chionaspis salicis-niger* Walsh. (2 slides)

Distribution: Russia (Saghalien: Horo).

Host plant: *Alnus hirsuta* (Betulaceae); *Salix* sp. (Salicaceae).

Syn.: *Coccus salicis* Linnaeus, 1758; *Coccus cryptogamus* Dalman, 1826; *Aspidiotes salicis* (Linnaeus 1758); *Aspidiotus Populi* Baerensprung, 1849; *Aspidiotus minimus* Baerensprung, 1849; *Diaspis nivea* Bremi, 1849; *Aspidiotus saliceti* Bouché, 1851; *Aspidiotus Vaccinii* Bouché, 1851; *Aspidiotus salicis-nigrae* Walsh, 1868; *Mytilaspis Maquarti* Targioni Tozzetti, 1868; *Mytilaspis cryptogama* (Dalman 1826); *Chionaspis aceris* Signoret, 1869; *Chionaspis alni* Signoret, 1869; *Chionaspis fraxini* Signoret, 1869; *Chionaspis vaccinii* (Linnaeus 1758); *Mytilaspis salicis* LeBaron, 1872; *Diaspis salicicorticis* Bessey, 1874; *Lecanium Vaccinii* (Bouché 1851); *Aspidiotus mytilus* Glaser, 1877; *Coccus* (*Aspidiotus*) *mytilus* (Glaser 1877); *Chionaspis sorbi* Douglas, 1893; *Chionaspis ortholobis bruneri* Cockerell, 1898; *Chionaspis salicis-nigrae* (Linnaeus 1758); *Aspidiotus cryptogamus* (Dalman 1826); *Chionaspis salicis* Archangelskaya, 1937; *Aspidiotus aceris* (Signoret 1869); *Aspidiotus alni* (Signoret 1869); *Chionaspis minimus* (Baerensprung 1849); *Aspidiotus salicifex* (Amyot 1847); *Chionaspis salicifex* (Amyot 1847); *Chionaspis montana* Borchsenius, 1949; *Chionaspis polypora* Borchsenius, 1949; *Chionaspis caucasioni* Hadzibejli, 1963.

***Chionaspis sozanica* Takahashi, 1933** (Fig. 1F) (Takahashi 1933; Tao 1989; Ben-Dov & Miller 2013)

Original spelling on label: *Chionaspis sozanicus* Takah. (3 slides)

Distribution: Taiwan (Taihoku: Sozan).

Host plant: *Acer* sp. (Aceraceae).

Syn.: *Trichomytilus sozanica* (Takahashi 1933); *Phenacaspis sozanica* (Takahashi 1933).

Note: Syntypes, female [collected from Taiwan: Sozan, near Taihoku, on *Acer* sp., 03-VII-1932, by R. Takahashi (#24-3-2-1–3)].

***Chionaspis trochodendri* (Takahashi 1936)** (Fig. 2A) (Takahashi 1936b; Takagi 1969; Chou 1982; Ben-Dov & Miller 2013)

Original spelling on label: *Phenacaspis trochodendri* Takah. (4 slides)

Distribution: Taiwan (Taihoku: Chikushiko, Sozan).

Host plant: *Trochodendron aralioides, Trochodendron* sp. (Trochodendraceae).

Syn.: *Phenacaspis trochodendri* Takahashi, 1936.

Note: Syntypes, female [collected from Taiwan: Chikushiko, near Sozan, Hichiseisan, Rarasan, near Urai, on *Trochodendron aralioides*, 25-XII-1935, by R. Takahashi (#40-1-1-1–40-1-1-4)].

***Duplachionaspis graminis* (Green 1896)** (Fig. 2B) (Green 1896; Ben-Dov & Miller 2013)

Original spelling on label: *Chionaspis graminis* Green. (8 slides)

Distribution: Taiwan (Taichu; Taihoku: Shinten, Taihoku, Tansui).

Host plant: *Miscanthus sinensis*, Poaceae, *Spinifex* sp. (Poaceae).

Syn.: *Chionaspis graminis* Green, 1896; *Trichomytilus graminis* (Green 1896); *Poliaspis graminis* (Green 1896).

***Duplachionaspis natalensis* (Maskell 1896)** (Fig. 2C) (Tao 1989; Ben-Dov & Miller 2013)

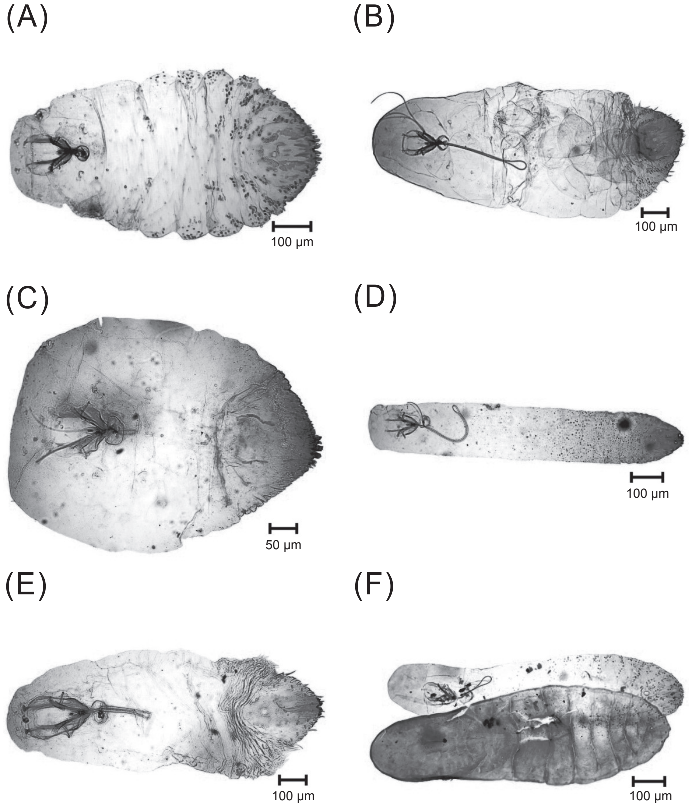

Fig. 2. (A) *Chionaspis trochodendri* (Takahashi 1936); (B) *Duplachionaspis graminis* (Green 1896); (C) *Duplachionaspis natalensis* (Maskell 1896); (D) *Greenaspis bambusifoliae* (Takahashi 1930); (E) *Greenaspis elongata* (Green 1896); and (F) *Kuwanaspis annandalei* (Green 1919).

Original spelling on label: *Chionaspis stanotophri* Cooley. (5 slides)

Distribution: Taiwan (Taihoku; Tainan; Taito: Kanoron).

Host plant: *Phragmites* sp., Poaceae, *Saccharum officinarum* (Poaceae).

Syn.: *Chionaspis spartinae natalensis* Maskell, 1896; *Chionaspis stanotophri* Cooley, 1899; *Chionaspis natalensis* Maskell, 1896; *Chionaspis* (*Phenacaspis*) *natalensis* (Maskell 1896); *Chionaspis graminis aegyptiaca* Hall, 1923; *Trichomytilus stanotophri* (Cooley 1899); *Polyaspis stanotophri* (Cooley 1899); *Poliaspis stanotophri* (Cooley 1899); *Duplachionaspis stanotophri* (Cooley 1899); *Duplachionaspis herbicola* Mamet, 1959.

***Greenaspis bambusifoliae* (Takahashi 1930)** (Fig. 2D) (Takahashi 1930; Tao 1989; Wong *et al.* 1999; Ben-Dov & Miller 2013)

Original spelling on label: *Chionaspis bambusifoliae* Takahashi. (11 slides)

Distribution: Taiwan (Nantou: Hori, Suisha; Taihoku).

Host plant: Bambusa sp. (Poaceae).

Syn.: Chionaspis bambusifoliae Takahashi, 1930.

Note: Syntypes, female [collected from Taiwan: Taihoku, on *Bambusa* sp., I-1930, by R.Takahashi (#23-2-2-1–2、#23-2-2-4–5)].

***Greenaspis elongata* (Green 1896)** (Fig. 2E) (Green 1896; Tao 1989; Wong *et al.* 1999; Ben-Dov & Miller 2013)

(1) **Original spelling on label:** *Chionaspis elongata* (Green 1896) (15 slides)

Distribution: Taiwan (Taihoku: Hichiseizan, Hokuto, Itabashi, Maruyama, Sozan, Taihoku; Taito: Riran).

(2) **Original spelling on label:** *Greenaspis elongata* Green, 1896 (1 slide)

Distribution: Malaysia (Malaya: Penang).

Host plant: *Bambusa* sp. (Poaceae).

Syn.: *Mytilaspis elongata* Green, 1896; *Chionaspis elongata* (Green 1896); *Trichomytilus elongatus* (Green 1896); *Greenaspis yunnanensis* Ferris, 1952.

***Kuwanaspis annandalei* (Green 1919)** (Fig. 2F) (Green 1919; Ben-Dov & Miller 2013)

Original spelling on label: *Chionaspis annandalei* Green. (1 slide)

Distribution: Taiwan (Giran: Suo).

Host plant: Poaceae.

Syn.: *Chionaspis annandalei* Green, 1919.

***Kuwanaspis arundinariae* Takahashi, 1938** (Fig. 3A) (Takahashi 1938; Tao 1989; Ben-Dov & Miller 2013)

Original spelling on label: *Kuwanaspis arundinariae* Takah. (4 slides)

Distribution: Taiwan (Giran: Taiheizan).

Host plant: *Arundinaria* sp. (Poaceae).

Syn.: *Nikkoaspis arundinariae* (Takahashi 1938).

Note: Syntypes, female [collected from Tai-

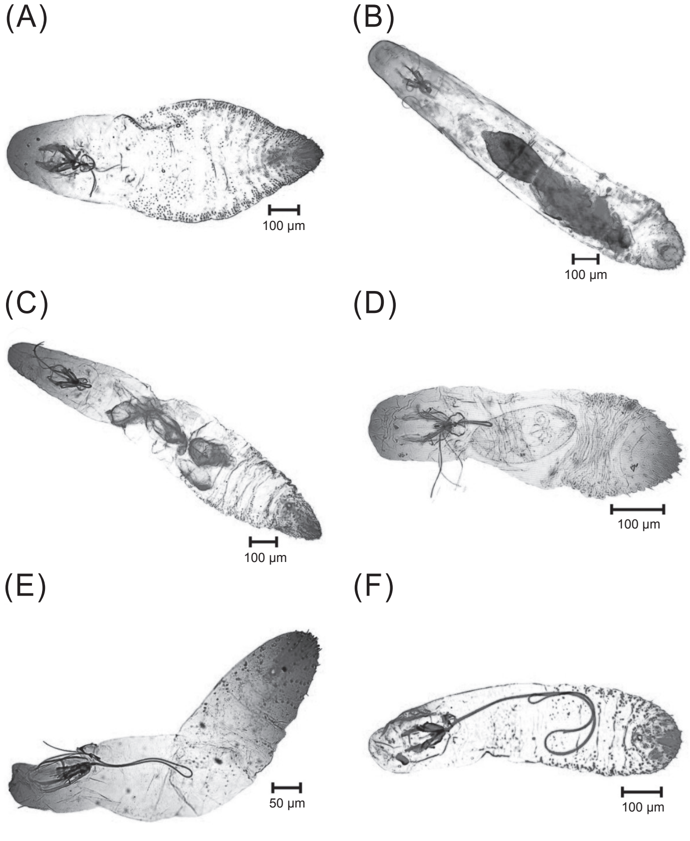

Fig. 3. (A) *Kuwanaspis arundinariae* Takahashi, 1938; (B) *Kuwanaspis bambusifoliae* (Takahashi 1934); (C) *Kuwanaspis elongata* (Takahashi 1930); (D) *Kuwanaspis linearis* (Green 1922); (E) *Kuwanaspis neolinearis* (Takahashi 1930); and (F) *Kuwanaspis phragmitis* (Takahashi 1931).

wan: Taiheizan, on *Arundinaria* sp., 14-X-1937 by R. Takahashi (#31-1-1-1–4)].

***Kuwanaspis bambusifoliae* (Takahashi 1934)** (Fig. 3B) (Takahashi 1934; Tao 1989; Ben-Dov & Miller 2013)

Original spelling on label: *Tsukushiaspis bambusifoliae* Takah. (2 slides)

Distribution: Taiwan (Taihoku: Hichiseizan).

Host plant: *Bambusa* sp. (Poaceae).

Syn.: *Tsukushiaspis bambusifoliae* Takahashi, 1934.

Note: Syntypes, female [collected from Taiwan: Taihoku, on Mt. Hichisei, on *Bambusa* sp., 27-III-1932 and 23-XI-1933, by R. Takahashi (#31-1-2-4–5)].

***Kuwanaspis elongata* (Takahashi 1930)** (Fig. 3C) (Takahashi 1930; Tao 1989; Wong *et al.* 1999; Ben-Dov & Miller 2013)

Original spelling on label: *Tsukushiaspis elongata* Takahashi. (6 slides)

Distribution: Taiwan (Nantou: Suisha; Taihoku: Maruyama).

Host plant: *Bambusa* sp., *Dendrocalamus* sp. (Poaceae).

Syn.: *Tsukushiaspis elongata* Takahashi, 1930.

Note: Syntypes, female [collected from Taiwan: Suisha, on *Bambusa* sp., 2-X-1929, by R. Takahashi (#31-1-3-1–5)].

***Kuwanaspis linearis* (Green 1922)** (Fig. 3D) (Takahashi 1942a; Chou 1982; Ben-Dov & Miller 2013)

Original spelling on label: *Kuwanaspis linearis* Green. (3 slides)

Distribution: Malaysia (Malaya: Kuala Lumpur); Singopore.

Host plant: *Bambusa* sp. (Poaceae).

Syn.: *Chionaspis linearis* Green, 1922; *Tsukushiaspis linearis* (Green 1922).

***Kuwanaspis neolinearis* (Takahashi 1930)** (Fig. 3E) (Takahashi 1930, 1942a; Tao & Wong 1982; Tao 1989; Wong *et al.* 1999; Ben-Dov & Miller 2013)

(1) **Original spelling on label:** *Chionaspis neolinearis* Takah. (2 slides)

Distribution: Taiwan (Nantou: Suisha).

Note: Syntype, female [collected from Taiwan: Taihoku, on *Bambusa* sp., 02-X-1929, by R. Takahashi (#23-5-1-2)].

(2) **Original spelling on label:** *Tsukushiaspis neolinearis* Takah. (12 slides)

Distribution: Singapore; Taiwan (Giran: Piyahau; Heito: Heito, Kuraru; Hualien: Karenko; Taihoku).

Host plant: *Bambusa* sp. (Poaceae).

Syn.: *Tsukushiaspis neolinearis* Takahashi, 1930; *Chuaspis neolinearis* (Takahashi 1930).

***Kuwanaspis phragmitis* (Takahashi 1931)** (Fig. 3F) (Takahashi 1931; Tao 1989; Ben-Dov & Miller 2013)

Original spelling on label: *Tsukushiaspis phragmitis* Takahashi. (6 slides)

Distribution: Taiwan (Giran: Suo; Taihoku: Sekinon, Tamsui; Taito: Chippon, Daichikko)

Host plant: *Artemisia feddei* (Compositae); *Phragmites* sp. (Poaceae).

Syn.: T*sukushiaspis phragmitis* Takahashi, 1931.

Note: Syntypes, female [collected from Taiwan: Suo, Chippon, Daichikko near Daibu, on *Phragmites* sp., 30-XII-1924, by R. Takahashi (#31-3-4-1–2)].

***Kuwanaspis pseudoleucaspis* (Kuwana 1902)** (Fig. 4A) (Kuwana 1902; Tao 1989; Ben-Dov & Miller 2013)

Original spelling on label: *Tsukushiaspis bambusae* Kuwana. (2 slides)

Distribution: Taiwan (Heito: Raisha).

Host plant: *Bambusa* sp. (Poaceae).

Syn.: *Leucaspis bambusae* Kuwana, 1902; *Lepidosaphes bambusae* (Kuwana 1902); *Mytilaspis bambusa*e (Kuwana 1902); *Chionaspis pseudoleucaspis* (Kuwana 1902); *Tsukushiaspis bam-*

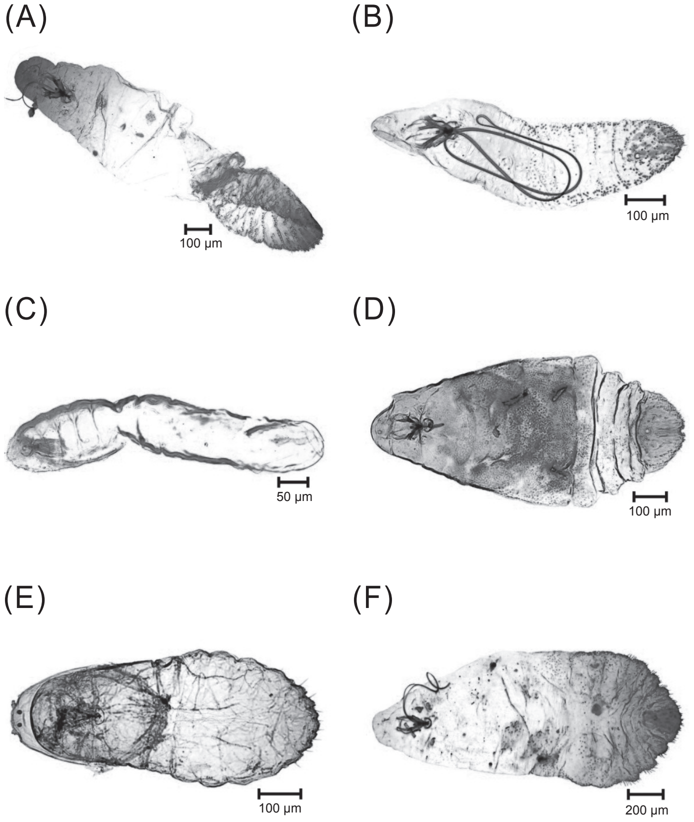

Fig. 4. (A) *Kuwanaspis pseudoleucaspis* (Kuwana 1902); (B) *Kuwanaspis suishana* (Takahashi 1930); (C) *Kuwanaspis vermiformis* (Takahashi 1931); (D) *Ledaspis atalantiae* (Takahashi 1933); (E) *Neoquernaspis lithocarpi* (Takahashi 1931); and (F) *Nikkoaspis formosana* (Takahashi 1930).

*busae (*Kuwana 1902); *Tsukushiaspis pseudoleucaspis* (Kuwana 1902); *Lepidosaphoides bambusae* (Kuwana 1902); *Kuwanaspis bambusae* (Kuwana 1902).

***Kuwanaspis suishana* (Takahashi 1930)** (Fig. 4B) (Takahashi 1930, 1942b; Tao 1989; Ben-Dov & Miller 2013)

Original spelling on label: *Tsukushiaspis shuishanus* Tak. (15 slides)

Distribution: Taiwan (Hualien: Karenko; Nantou: Suisha; Taihoku: Maruyama, Taihoku); Thailand (Chingmai).

Host plant: *Bambusa* sp., *Bambusa stenostachya*. (Poaceae).

Syn.: *Tsukushiaspis suishanus* Takahashi, 1930; *Kuwanaspis suishanus* (Takahashi 1930).

***Kuwanaspis vermiformis* (Takahashi 1931)** (Fig. 4C) (Takahashi 1931, Tao 1989; Ben-Dov & Miller 2013)

Original spelling on label: *Tsukushiaspis vermiformis* Takah. (4 slides)

Distribution: Taiwan (Taihoku: Maruyama, Taihoku).

Host plant: *Bambusa* sp., *Bambusa stenostachya*, *Dendrocalamus latiflorus*. (Poaceae).

Syn.: *Tsukushiaspis vermiformis* Takahashi, 1931.

Note: Syntypes, female [collected from Taiwan: Taihoku, Tosei, on *Bambusa stenostachya*, *Bambusa* sp., *Dendrocalamus latiflorus* (#31-5-3-1–3)].

***Ledaspis atalantiae* (Takahashi 1933)** (Fig. 4D) (Takahashi 1933; Ben-Dov & Miller 2013)

(1) **Original spelling on label:** *Chionaspis atalantiae* Takah. (7 slides)

Distribution: Taiwan (Heito: Kontei; Takao).

Note: Syntypes, female [collected from Taiwan: Kontei, near Koshun, on *Atalantia lilocularis*, 24-V-1932, by R.Takahashi (#23-1-2-1–23-1-3-1)].

(2) **Original spelling on label:** *Dinaspis atalantiae* Takah. (4 slides)

Distribution: Taiwan (Heito: Garanbi).

Host plant: *Atalantia lilocularis*, *Severinia buxifolia* (Rutaceae).

Syn.: *Chionaspis atalantiae* Takahashi, 1933; *Trichomytilus atalantiae* (Takahashi 1933); *Phenacaspis atalantiae* (Takahashi 1933); *Pseudaulacaspis atalantiae* (Takahashi 1933).

***Neoquernaspis lithocarpi* (Takahashi 1931)** (Fig. 4E) (Takahashi 1931; Tao 1989; Ben-Dov & Miller 2013)

Original spelling on label: *Pinnaspis lithocarpi* Takah. (6 slides)

Distribution: Taiwan (Nantou: Hori, Suisha).

Host plant: *Lithocarpus* sp., *Pasania ternaticupula*. (Fagaceae)

Syn.: *Pinnaspis lithocarpi* Takahashi, 1931; *Quernaspis lithocarpi* (Takahashi 1931).

Note: Syntypes, female [collected from Taiwan: Suisha, on *Lithocarpus* sp., 23-XI-1929, by R. Takahashi (#41-3-3-1–2)].

***Nikkoaspis formosana* (Takahashi 1930)** (Fig. 4F) (Takahashi 1930, 1934; Tao 1989; Ben-Dov & Miller 2013)

Original spelling on label: *Tsukushiaspis formosanus* Takah. (5 slides)

Distribution: Taiwan (Nantou: Suisha).

Host plant: *Bambusa* sp. (Poaceae).

Syn.: *Tsukushiaspis formosanus* Takahashi, 1930; *Kuwanaspis formosana* (Takahashi 1930).

Note: Syntypes, female [collected from Taiwan: Suisha, on *Bambusa* sp., 23-XI-1929 (#31-2-1-1–2) and 26-I-1930 (#31-2-1-4–5) by R. Takahashi].

***Nikkoaspis hichiseisana* (Takahashi 1934)** (Fig. 5A) (Takahashi 1934; Takagi 1961; Tao 1989; Ben-Dov & Miller 2013)

Original spelling on label: *Tsukushiaspis hichiseisana* Takah. (8 slides)

Distribution: Taiwan (Taihoku: Hichiseizan).

Host plant: *Bambusa* sp. (Poaceae).

Syn.: T*sukushiaspis hichiseisana* Takahashi, 1934; *Kuwanaspis hichiseisana* (Takahashi, 1934).

Note: Syntypes, female [collected from Taiwan: Mt. Hichisei, near Taihoku, on *Bambusa* sp., 23-IX-1933 by R. Takahashi (#31-2-2-1–31-2-3-3)].

***Nikkoaspis sasae* (Takahashi 1936)** (Fig. 5B) (Takahashi 1936a; Takagi 1961; Chou 1982; Ben-Dov & Miller 2013)

Original spelling on label: *Tsukushiaspis sasae* Tak. (3 slides)

TARI specimens Distribution: China (Zhejiang: Hangzhou, Huchow).

Host plant: *Bambusa* sp., *Sasa* sp. (Poaceae).

Syn.: *Tsukushiaspis sasae* Takahashi, 1936; *Kuwanaspis sasae* (Takahashi 1936).

Note: Syntypes, female [collected from China: Zhejiang, Huchow, on *Sasa* sp., VIII-1935, by S.P. Chu (#31-4-2-1–2)].

***Nikkoaspis shiranensis* Kuwana, 1928** (Fig. 5C) (Takahashi 1934; Ben-Dov & Miller 2013)

Original spelling on label: *Kuwanaspis shiranensis* Kuwana. (1 slide)

Distribution: Japan (Hokkaido: Sapporo).

Host plant: *Sasa veitchii* (Poaceae).

Syn.: *Tsukushiaspis shiranensis* (Kuwana 1928); *Kuwanaspis shiranensis* (Kuwana 1928).

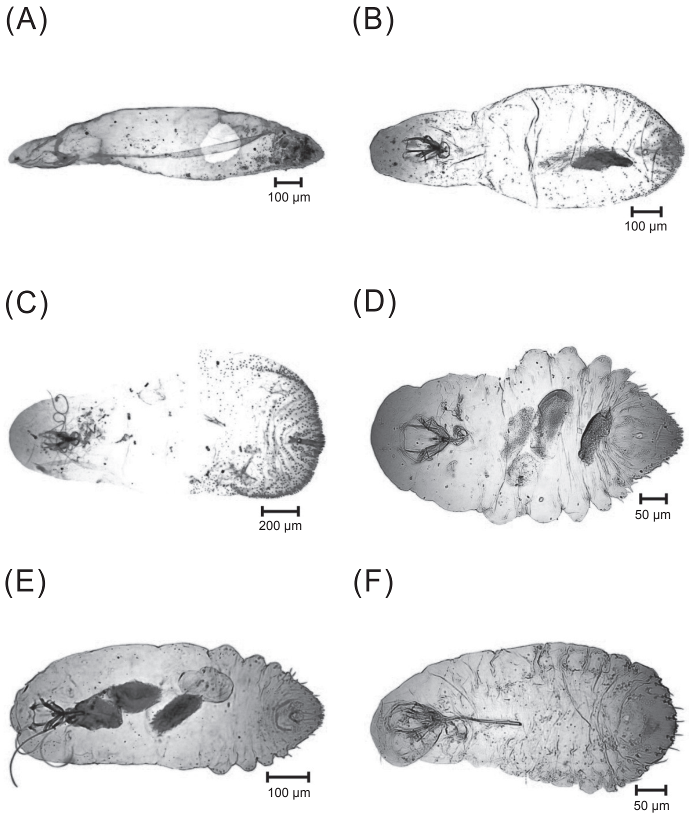

Fig. 5. (A) *Nikkoaspis hichiseisana* (Takahashi 1934); (B) *Nikkoaspis sasae* (Takahashi 1936); (C) *Nikkoaspis shiranensis* Kuwana, 1928; (D) *Pinnaspis aspidistrae aspidistrae* (Signoret 1869); (E) *Pinnaspis javanensis* (Kuwana in Kuwana & Muramatsu 1931); and (F) *Pinnaspis siamensis* Takahashi, 1942.

***Pinnaspis aspidistrae aspidistrae* (Signoret 1869)** (Fig. 5D) (Lindinger 1912; Tao 1989; Wong *et al.* 1999; Ben-Dov & Miller 2013)

(1) **Original spelling on label:** *Hemi-Chionaspis aspidistrae* Signt. (1 slide)
Distribution: Taiwan (Taihoku).

(2) **Original spelling on label:** *Pinnaspis aspidistrae* Signt. (44 slides)
Distribution: China (Foochow); Fiji; Japan (Kyushu: Kagoshima); Okinawa; Malaysia (Malaya: Bukit Asahan, G. Batu Brinchang, Kuala Lumpur, Serdang, Taiping); Taiwan (Giran: Churei; Heito: Garanbi; Hualien: Karenko; Nantou: Habon Near Urai, Suisha; Taihoku: Hinokiyama, Shirin, Taihoku; Tainan; Taito: Kaede, Kakayo, Oiwake; Takaoshu: Hinode); Thailand (Ubon).

Host plant: *Asplenium* sp. (Aspleniaceae); *Citrus* sp. (Rutaceae); *Cyathea* sp., *Cycas* sp. (Cycadaceae); *Cymbidium ensifolium*, Orchid (Orchidaceae), *Daemonorops* sp., *Elaeis guineensis* (Arecaceae); *Diospyros* sp. (Ebenaceae); *Elatostema* sp. (Urticaceae); *Fern* sp. (Psilotaceae); *Ficus* sp., *Ficus pumila*, *Ficus retusa*, *Ficus tinctoria* (Moraceae); *Hydrangea* sp. (Saxifragaceae); *Pandanus* sp., *Pandanus atrocarpus* (Pandanaceae); *Podocarpus* sp. (Podocarpaceae), *Rhododendron probably jasminiflorum* (Ericaceae); *Smilax* sp. (Smi-

lacaceae); *Styrax* sp. (Styracaceae); *Viburnum* sp. (Caprifoliaceae).

Syn.: *Chionaspis aspidistrae* Signoret, 1869; *Chionaspis brasiliensis* Signoret, 1869; *Chionaspis latus* Cockerell, 1896; *Hemichionaspis aspidistrae brasiliensis* (Signoret 1869); *Hemichionaspis aspidistrae lata* (Signoret 1869); *Chionaspis* (*Pinnaspis*) *aspidistrae* (Signoret 1869); *Pinnaspis* (*Hemichionaspis*) *aspidistrae* (Signoret 1869); *Pinnaspis brasiliensis* (Signoret 1869); *Pinnaspis ophiopogonis* Takahashi, 1952; *Pinnaspis caricis* Ferris, 1957.

Pinnaspis javanensis **(Kuwana 1931)** (Fig. 5E) (Takagi 1985; Ben-Dov & Miller 2013)

Original spelling on label: *Pinnaspis javanensis* Kuwana. (1 slide)

Distribution: Malaysia (Malaya: Sunge Buloh).

Host plant: *Hevea* sp. (Euphorbiaceae).

Syn.: *Chionaspis javanensis* Kuwana, 1931.

Pinnaspis siamensis **Takahashi, 1942** (Fig. 5F) (Takahashi 1942b; Ben-Dov & Miller 2013)

Original spelling on label: *Pinnaspis lithocarpi* var. *siamensis* Tak. (8 slides)

Distribution: Thailand (Mt. Sutep; Siam).

Syn.: *Pinnaspis lithocarpi siamensis* Takahashi, 1942; *Quernaspis siamensis* (Takahashi 1942).

Host plant: *Lithocarpus* sp. (Fagaceae)

Note: Syntypes, female [collected from Thailand: Mt. Sutep, on *Lithocarpus* sp. 09-IV-1940, by R. Takahashi (#41-4-1-1–41-4-2-3)].

Pinnaspis strachani **(Cooley 1899)** (Fig. 6A) (Tao 1989; Ben-Dov & Miller 2013)

(1) **Original spelling on label:** *Hemichionaspis minor* Mask. (1 slide)

Distribution: Taiwan (Taihoku: Taihoku).

(2) **Original spelling on label:** *Pinnaspis minor* Mask. (12 slides)

Distribution: French Polynesia (Tahiti); Micronesia (Caroline Isls.: Ponape: Kolonia); Taiwan (Heito: Koshun; Taihoku: Hichiseizan; Taoyuan: Kaho); Thailand (Bangkok).

Host plant: *Chelidonium* sp. (Papaveraceae); *Cocos nucifera* (Arecaceae); *Eurya* sp. (Theaceae); *Planchonella* sp. (Sapotaceae); *Solanum* sp. (Solanaceae).

Syn.: *Hemichionaspis minor strachani* Cooley, 1899; *Hemichionaspis Marchali* Cockerell, 1902; *Hemichionaspis townsendi* Cockerell, 1905; *Chionaspis* (*Hemichionaspis*) *aspidistrae gossypii* Newstead, 1908; *Hemichionaspis aspidistrae gossypii* (Newstead 1906); *Hemichionaspis proxima* Leonardi, 1914; *Hemichionaspis* (*Pinnaspis*) *marchali* (Cockerell 1902); *Pinnaspis minor* (Cooley 1899); *Pinnaspis minor strachani* (Cooley 1899); *Pinnaspis proxima* (Leonardi 1914); *Pinnaspis* (*Hemichionaspis*) *aspidistrae gossypii* (Newstead 1908); *Pinnaspis temporaria* Ferris, 1942; *Pinnaspis aspidistrae gossypii* (Newstead 1908); *Pinnaspis gossypii* (Newstead 1908); *Pinnaspis marchali* (Cockerell 1902); *Hemichionaspis gossypii* (Newstead 1908); *Chionaspis* (*Hemichionaspis*) *gossypii* (Newstead 1908); *Pinnaspis townsendi* (Cockerell 1905).

Pinnaspis theae **(Maskell 1891)** (Fig. 6B) (Takahashi 1929; Tao 1989; Ben-Dov & Miller 2013)

Original spelling on label: *Pinnaspis theae*

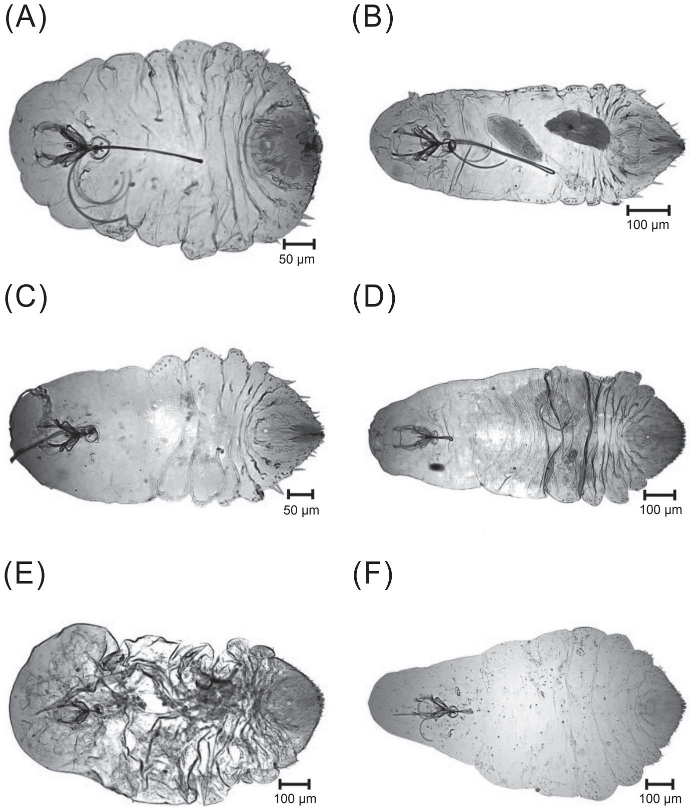

Fig. 6. (A) *Pinnaspis strachani* (Cooley 1899); (B) *Pinnaspis theae* (Maskell 1891); (C) *Pinnaspis uniloba* (Kuwana 1909); (D) *Unaspis acuminata* (Green 1896); (E) *Unaspis citri* (Comstock 1883); and (F) *Unaspis euconymi* (Comstock 1881).

Mask. (3 slides)

Distribution: Taiwan (Heito: Koshun; Tainan: Kanshirei; Takao).

Host plant: *Cleyera japonica*, *Thea japonica*, *Thea* sp. (Theaceae).

Syn.: *Chionaspis theae* Maskell, 1891; *Aspidiotus theae* (Maskell 1891); *Chionaspis aspidistrae theae* (Maskell 1891); *Hemichionaspis theae* (Maskell 1891); *Chionaspis separata* Green, 1900; *Hemichionaspis separata* (Green 1900); *Trichomytilus theae* (Maskell 1891); *Pinnaspis separata* (Green 1900).

***Pinnaspis uniloba* (Kuwana 1909)** (Fig. 6C)
(Ferris 1921; Takahashi 1929; Tao 1989; Wong *et al.* 1999; Ben-Dov & Miller 2013)

(1) **Original spelling on label:** *Pinnaspis simplex* Ferris. (3 slides)

Distribution: Taiwan (Tainan: Kanshirei, Sekitei).

(2) **Original spelling on label:** *Pinnaspis uniloba* Kuwana. (1 slide)

Distribution: Taiwan (Taihoku: Sozan).

Host plant: *Camellia* sp., *Cleyera japonica* (Theaceae).

Syn.: *Mytilaspis* (*Lepidosaphes*) *uniloba* Kuwana, 1909; *Lepidosaphes uniloba* (Kuwana 1909); *Jaapia uniloba* (Kuwana 1909); *Lepidaspidis uniloba* (Kuwana 1909); *Pinnaspis simplex* Ferris, 1921.

***Unaspis acuminata* (Green 1896)** (Fig. 6D)

(Green 1896; Chou 1982; Ben-Dov & Miller 2013)

Original spelling on label: *Unaspis acuminata* Green. (2 slides)

Distribution: Thailand (Chiengsen; Mt. Sutep).

Host plant: *Mangifera indica* (Anacardiaceae).

Syn.: *Chionaspis acuminata* Green, 1896.

***Unaspis citri* (Comstock 1883)** (Fig. 6E) (Chou 1982; Ben-Dov & Miller 2013)

(1) **Original spelling on label:** *Dinaspis veitchi* Green et Laing. (1 slide)

Distribution: Papua New Guinea (Admiralty Isls.: Manus).

(2) **Original spelling on label:** *Prontaspis citri* Comst. (1 slide)

Distribution: Malaysia (Malaya).

Host plant: *Citrus* sp. (Rutaceae).

Syn.: *Chionaspis citri* Comstock, 1883; *Howardia citri* (Comstock 1883); *Chionaspis citricola* (Comstock 1883); *Dinaspis annae* Malenotti, 1916; *Prontaspis citri* (Comstock 1883); *Dinaspis veitchi* Green & Laing, 1923; *Trichomytilus veitchi* (Green & Laing 1923); *Diaspis annae* (Malenotti 1916); *Chionaspis annae* (Malenotti 1916).

***Unaspis euconymi* (Comstock 1881)** (Fig. 6F) (Chou 1982; Ben-Dov & Miller 2013)

Original spelling on label: *Unaspis nakayamai* Takah. et Kanda. (1 slide)

Distribution: Korea (Suigen).

Host plant: *Ilex serrata* (Aquifoliaceae).

Syn.: *Chionaspis euonymi* Comstock, 1881; *Unaspis euonymi* (Comstock 1881); *Unaspis nakayamai* Takahashi & Kanda, 1939.

***Unaspis kanoi* (Takahashi 1935)** (Fig. 7A) (Takahashi 1935b; Ben-Dov & Miller 2013)

Original spelling on label: *Lepidosephas* (*Coccomytilus*) *kanoi* Takah. (5 slides)

Distribution: Taiwan (Taito: Kotosho).

Syn.: *Coccomytilus kanoi* Takahashi, 1935.

Note: Syntypes, female [collected from Taiwan: Botel Tobago, on undetermined tree, 1935, by T. Kano (#33-3-4-1–5)].

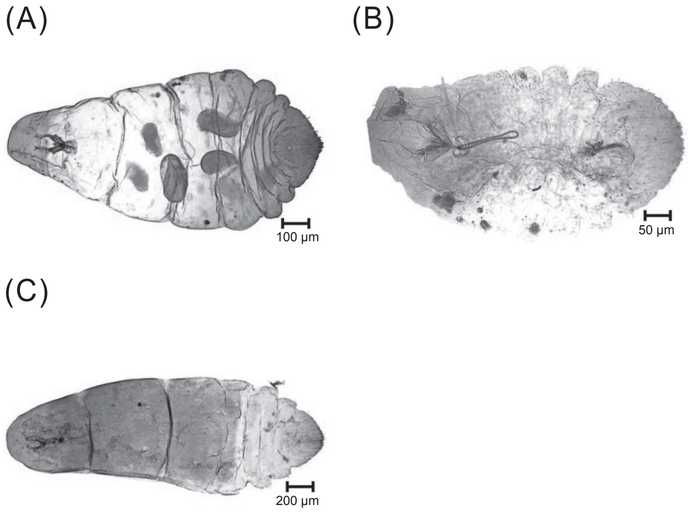

Fig. 7. (A) *Unaspis kanoi* (Takahashi 1935); (B) *Unaspis turpiniae* Takahashi, 1934; and (C) *Unaspis yanonensis* (Kuwana 1923).

***Unaspis turpiniae* Takahashi, 1934** (Fig. 7B)
(Takahashi 1934; Tao 1989; Ben-Dov & Miller 2013)

Original spelling on label: *Dinaspis acuminata var. turpiniae* Takah. (2 slides)
Distribution: Taiwan (Giran: Muroruafu).
Host plant: *Ilex* sp. (Aquifoliaceae).
Syn.: *Unaspis acuminata turpiniae* Takahashi, 1934; *Chionaspis turpinae* (Takahashi 1934).

***Unaspis yanonensis* (Kuwana 1923) (**Fig. 7C)
(Takahashi & Kanda, 1939; Chou 1982; Ben-Dov & Miller 2013)

Original spelling on label: *Prontaspis yanonensis* Kuw. (2 slides)
Distribution: Taiwan (Taihoku: Sankyo near Taihoku).
Host plant: *Citrus* sp. (Rutaceae).
Syn.: *Prontaspis yanonensis* Kuwana, 1923.

DISCUSSION

Based on the modern taxonomic system, the number of Chionaspinae species collected and deposited at TARI Insect Collection is 39. The present paper could be a database to set up the online information of scientific name, synonyms, host plants, distribution and digital archives through Digital Insect of TARI website (http://digiins.tari.gov.tw/) which will meet the requirement of both basic and applied works and will be helpful to taxonomic researchers, quarantine inspectors and farmers in the future.

ACKNOWLEDGMENTS

We would like to thank the National Science Council (grant numbers 99-2631-H-055-001 and 100-2631-H-055-001) and Council of Agriculture, Executive Yuan (grant number 102AS-7.1.1-CI-C1) for financial support.

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農業試驗所昆蟲標本館雪盾介殼蟲亞科 (半翅目：胸喙亞目：介殼蟲總科：盾介殼蟲科) 標本名錄

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摘要

陳淑佩、翁振宇、吳文哲。2014。農業試驗所昆蟲標本館雪盾介殼蟲亞科 (半翅目：胸喙亞目：介殼蟲總科：盾介殼蟲科) 標本名錄。台灣農業研究 63(2):105–121。

本文羅列 9 屬 39 種 (246 片玻片標本) 農業試驗所館藏之雪盾介殼蟲亞科 [半翅目 (Hemiptera) 胸喙亞目 (Sternorrhyncha) 介殼蟲總科 (Coccoidea) 盾介殼蟲科 (Diaspididae)] 名錄，其中亦包含異名、寄主植物、採集分佈及標本形態圖等資料。

關鍵詞：盾介殼蟲、農業試驗所、館藏標本。

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台灣農業研究 (J. Taiwan Agric. Res.) 63(2):122–128 (2014)
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「台農 67 號」穀粒充實過程動態影像變化之研究

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摘要

林資哲、劉威廷、黃國益、宋濟民、李長沛、吳東鴻、賴明信。2014。「台農 67 號」穀粒充實過程動態影像變化之研究。台灣農業研究 63(2):122–128。

本研究開發一套軟體針對水稻不同穀粒充實期外觀進行影像分析，期以瞭解其動態變化。研究係以「台農 67 號」品種為材料，在抽穗後 7、14、21、28、35、42 d 進行取樣，收集穗梢往穗基之 5 個一次枝梗，對位於由小支梗尖部至基部的第 2、3、4、5 位置穀粒進行粒形的影像掃描及分析。結果顯示，穀粒粒形變化以內穎變化較外穎明顯，外穎部分在抽穗之後的變化並不大。而充實期穀粒外形輪廓的變化，主要在於穀粒之厚度，在抽穗 7 d 後快速增加，其中內穎部分以掃描 105 度至 150 度之間變化最大，且會隨著充實時間的增加會有增厚的情形。利用影像軟體進行穀粒外形輪廓掃描，是一種新型高效率的分析方法，能夠清楚描述穀粒充實期外觀之變化。

關鍵詞：水稻、「台農 67 號」、穀粒充實期、一次枝梗、影像分析。

前言

水稻是全世界三大作物之一，以水稻爲主食的人口數則是全球第一。台灣地處熱帶至亞熱帶之間，氣候土壤適合水稻生長，乃台灣栽培面積最廣且最重要的糧食作物。近年來政府爲提昇國產稻米品質，致力於良質米推薦品種稻種之供應，而優良且純一稻種的推廣及更新，關係這項工作的成敗。除了仰賴育種者不斷選育優良新品種外，如何快速而且準確的判別稻種純度技術之建立更是不可或缺。傳統水稻穀粒的檢測方法依賴人力，無法詳細的以肉眼進行辨別，若能藉助立體顯微鏡將穀粒做細微觀察，並以影像處理系統將外形特徵數量化，將可作爲客觀而快速判別的依據 (Chou *et al*. 2011)。近年來數位化影像處理技術發展迅速，已逐步的使用於農業自動化之研究，如將穀粒特徵與數位化影像處理結合，則有助於穀粒之判別又可加速檢驗之流程，使育種人員快速有效檢控品種之純度，並可提升水稻育種程序中穀粒性狀之評估效率。Travis & Draper (1985) 利用影像處理系統量測 49 種穀粒及雜草種子的長度和外型，發現其量測結果可達到分辨之效果。Chang *et al*. (2000) 利用紅外線及影像外觀特徵值取面積、周長、形狀、面積/周長、最大寬度值、最長軸等參數值進行 6 個水稻品種鑑別，其鑑別率可達 65.4% 以上。Tanabata *et al*. (2010) 利用影像處理技術快速有效量測植物器官的形狀及開花時期花冠面積變化情形，並可有效的辨別開花時期花色變化與老化間之關係。Tanabata *et al*. (2012) 利用影像程式快速量測水稻品種 ‘Koshihikari’ 和 ‘Nipponbare’ 成熟種子的長度、寬度、周長等

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參數，可以有效快速的測量成熟種子之參數，測量結果並可以有效提供給育種人員利用。Lee *et al*. (2011) 利用電腦影像分析種植在韓國 7 種不同穀類種子，並分別對穀粒彩色圖像作數據收集，針對穀粒的 7 種顏色和外觀形態特徵的進行判別，分析結果可以有效提高辨識效率。

水稻穀粒的形狀及大小是具遺傳性的農藝性狀，它會影響產量和市場之價格 (Tanabata *et al*. 2012; Wang *et al*. 2012)。水稻穀粒形狀複雜而不明確，缺乏精確的定義，一般以穀粒長寬比例來加以描述 (Lu *et al*. 2003)，如何獲得準確的穀粒大小數據，俾以快速大量的測量有其實用價值。本研究材料「台農 67 號」是由行政院農業委員會農業試驗所登記命名，為 1967 年至 1987 年的領先品種；品種特性為強桿、不易倒伏、適合機械收穫、具有穩定高產潛力、適應性廣，對病蟲害之忍耐性強等，為台灣地區主要水稻栽培之對照品種 (Hwang 1987)。因此，本研究擬藉由影像處理系統調查「台農 67 號」，在不同穀粒充實階段之粒長、粒寬、粒厚及粒長與粒寬比例之變化，探討穀粒充實過程外表形態改變之情形。

材料與方法

本研究以水稻「台農 67 號」(‘Tainung67’ or ‘TNG67’) 品種作為試驗材料，於 2012 年第二期作種植於台中市霧峰區國立中興大學農業試驗場。在抽穗前掛牌標定共 70 枝單穗，於抽穗後第 7、14、21、28、35、42 d 進行試驗材料取樣 (n = 10)，並秤其鮮重後置於 60℃烘箱，烘乾 72 h 後秤其乾物重。強勢粒取樣是參考 Ishimaru *et al*. (2003) 進行，收集穗梢往穗基之 5 個一次枝梗，對位於由小支梗尖部至基部的第 2、3、4、5 位置之穀粒取樣後秤其重量，放入 FAA (formol-acetic acid-alcohol) 固定液 (75% ethanol : formalin : glacial acid = 90 : 5 : 5, v/v/v) (Hong *et al*.1996) 中固定2 d，取出穀粒進行影像拍攝。正面影像分析先利用影像程式描繪出穀粒輪廓，並檢測出穀粒之最長軸，由長軸之中心點位子，每 0.5 度從內穎向稃尖進行掃描，並記錄從 0 至 180 度內穎變化，及記錄從 180 度至 360 度外穎變化之情形。側面影像分析是由長軸之中心點位子，每 0.5 度由護穎向稃尖進行 360 度掃描並進行紀錄。影像拍攝設備為 UI-2280SE (IDS，Germany) 之 CCD，鏡頭為 ML-3519 (MORITEX，Japan)。

結果

充實時期之穀粒外形變化

本試驗以「台農 67 號」為材料，於 2012 年 8 月種植至田間，種植後 72 d ± 2 d 開始抽穗，充實時期穀粒外形的動態變化如圖 1A、1B，穗之鮮重、乾重及強勢粒重量的變化如圖 1C。結果在圖 1A、1B 觀察到「台農 67 號」抽穗後 7 d，穀粒外形輪廓尚未有膨脹情形，穀粒外形輪廓在抽穗後 14 d 開始有產生膨大情形，且抽穗 14 d 時穀粒之厚度有明顯增加，並會隨著充實日數的增加穀粒厚度會有增厚的情形，且在抽穗 28 d 後穀粒顏色也由綠色轉變成金黃色。穀粒充實時期穗的鮮重及乾重變化如圖 1C，在充實 42 d 時平均穗重的鮮重及乾重達到最重，分別為 4.82 g 及 3.36 g。在不同穀粒充實時期的強勢粒重量的變化，以穀粒充實時 35 d 的平均強勢粒重量 0.45 g 為最重，其次為充實時 42 d 0.440 g，充實時 7 d 0.027 g 最輕。

充實時期穀粒之輪廓變化

取「台農 67 號」在不同充實時期之穀粒，以 CCD 攝影設備進行取像，經影像程式去除芒及小枝梗後，進行影像分析，穀粒平面影像從 0 度至 180 度為內穎的範圍、180 至 360 度為外穎的範圍。圖 2A 及 2B 為穀粒充實時，平面和側面影像之掃描圖，而圖 2C 及 2D 為不同充實時期，穀粒外形的平面及側面變化曲線。穀粒平面變化曲線圖 (2C) 顯示，內穎長度在穀粒充實 21 d 後有明顯增加，內穎長度較其他生育期之內穎長度長，穀粒外形掃描曲線在 40 度至 120 度部位的長度變化，明顯長於其他部位；但不同充實時期之穀粒外穎長度變化並不明顯。由掃描的結果得知，不同充實時期穀粒平面外觀輪廓變化情形，以內穎變化較外穎明顯；外穎部分在抽穗之後的變化並沒

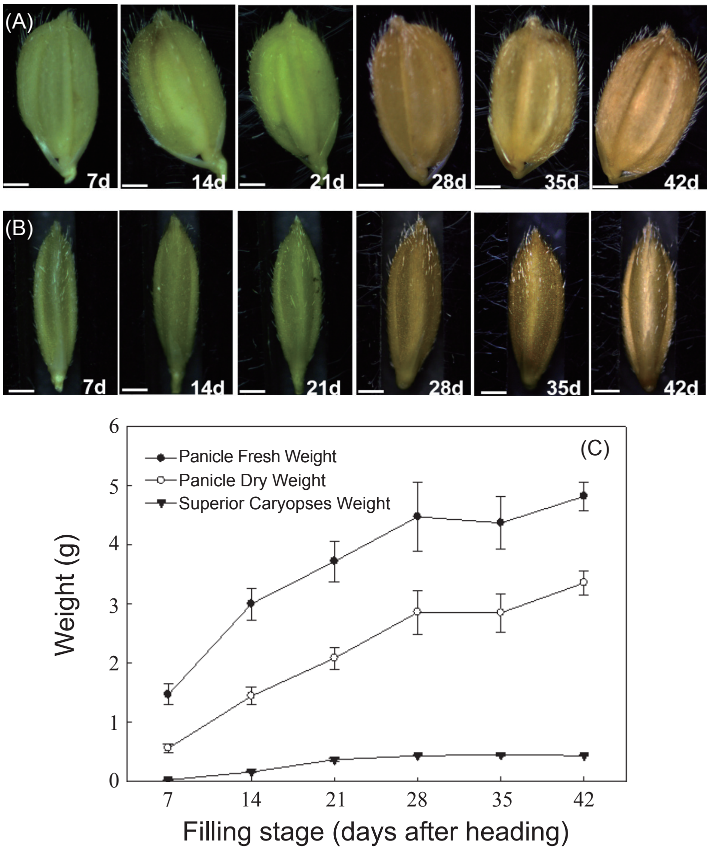

圖 1.　「台農 67 號」穀粒充實期間粒形變化情形。(A) 平面圖；(B) 側面圖；(C) 乾物重。

Fig. 1.　The images were shown according to the time courses during grains filling stage of rice cultivar 'Tainung67'. (A) surface-view; (B) lateral-view; and (C) biomass accumulation. (bar = 1 mm)

有太大的差異。而不同穀粒充實時期的側面輪廓外觀變化如圖 2D，不同穀粒充實程度在抽穗 7 d 後，穀粒會隨著充實日數的增加內外穎的厚度會有增加的情形，其中以充實 28 d 增加較為明顯。本試驗結果顯示了不同充實期穀粒外觀輪廓的變化，均以穀粒中心點到邊緣的直線距離的量測結果來表示，由圖 2E 顯示穀粒充填過程，內穎擴充增大的幅度明顯大過外穎；大約在 10 度至 50 度之間，隨著穀粒充實過程，是內穎擴充增大幅度最明顯的範圍。而穀粒側面變化，由圖 2F 顯示約在 105 度至 150 度之間，穀粒的厚度會隨穀粒充實日數的延長，增加則最為明顯。

充實時期穀粒之粒形參數變化

將不同充實時期之穀粒，利用影像掃描分析 7、14、21、28、35 及 42 d 穀粒之長度、寬度、厚度、長寬度比值等參數，其結果如表 1 所示。不同充實時期穀粒水平掃描後，穀粒長度介於 7.00–7.38 mm 之間，穀粒充實至 35 d 時最長，粒長為 7.38 mm，穀粒在充實 42 d 時穀粒長度有縮短的情形產生。不同充實時期，穀粒寬度變化介於 3.50–3.97 mm 之間，以充實 35 d 寬度可達 3.97 mm 最寬，充實 28 d 後穀粒之寬度變化則無明顯差異。不同充實時期穀粒側面掃描後，穀粒厚度介於 1.72–2.56 mm 間，隨著穀粒充實時間的增加，以充實 35 d 種子厚度可達 2.56 mm 最厚。不同充實時期穀粒長寬比值介於 1.82–2.00 之間，以充實 7 d 長寬比值為 2.00 最大，而充實時 42 d 長寬比值 1.82 較小。而且不同充實時期穀粒長寬

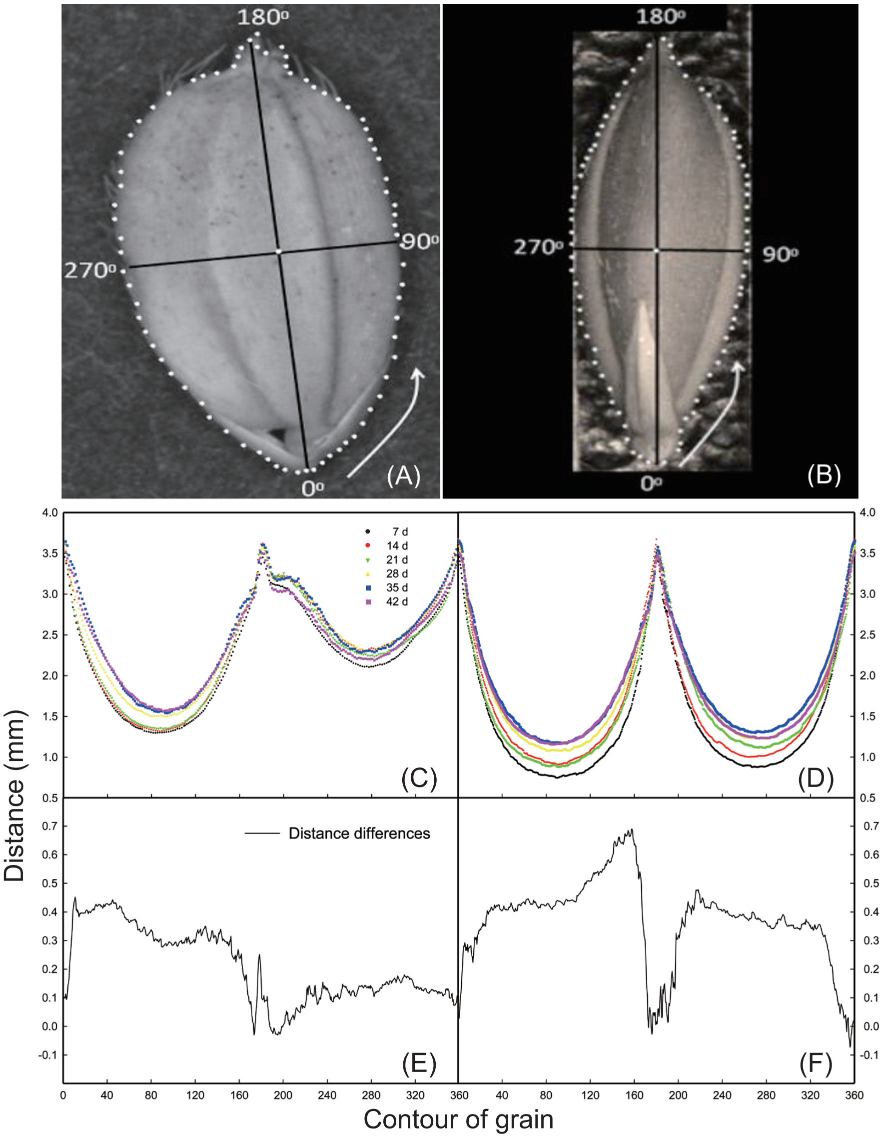

圖 2. 影像軟體分析穀粒表面及側面之情形。(A) 平面影像；(B) 側面影像；(C) 分析後表面圖像輸出；(D) 分析後側面圖像輸出；(E) 平面圖像充實期間變化量；(F) 側面圖像充實期間變化量。

Fig. 2. The software can analyze both surface and lateral image of grain automatically. (A) Surface image; (B) lateral image; (C) output for the analysed surface image; (D) output for the analysed lateral image; (E) distance differences of surface image during filling period; and (F) distance differences of lateral image during filling period.

比值的變化，會隨著抽穗期日數的增加，比值會有降低的情形。

討論

水稻穀粒外形變化的同時，其內部也在同步進行充實與成熟。一般水稻穀粒的成熟初期顏色為綠色，待成熟時會轉為金黃色。而穀粒成熟期間質地也由乳狀、糊狀而轉變為成熟時堅硬的質地。因此，水稻穀粒的成熟過程，可分為乳熟、黃熟、完熟及枯熟幾個時期 (Matsuo *et al*.1993)。穀粒是稻穗重要的組成性狀之一，更是水稻結實過程中同化產物的接收器

表 1. 「台農 67 號」穀粒充實期間穀粒最長的長度 (A)、最長的寬度 (B)、穀粒厚度及穀粒之長寬比之情形。

Table 1. During the filling stage, the longest length (A), longest width (B), longest thickness and ratio of A to B for rice cultivar 'Tainung67'[z].

Filling stage (days after heading)	Longest length (A) (mm)	Longest width (B) (mm)	Longest thickness (mm)	A/B
7	7.00 ± 0.13 b	3.50 ± 0.19 c	1.72 ± 0.16 e	2.00
14	7.34 ± 0.20 a	3.76 ± 0.13 b	2.01 ± 0.06 d	1.95
21	7.35 ± 0.21 a	3.73 ± 0.13 b	2.16 ± 0.14 c	1.97
28	7.26 ± 0.22 a	3.96 ± 0.11 a	2.41 ± 0.08 b	1.83
35	7.38 ± 0.30 a	3.97 ± 0.14 a	2.56 ± 0.13 a	1.85
42	7.14 ± 0.12 ab	3.92 ± 0.14 a	2.50 ± 0.09 ab	1.82

[z] Means ± standard errors (n = 10) within each column followed by different letters are significantly different at the $P = 0.05$ level.

官，在生產上直接影響收穫的經濟產量 (Liang *et al*. 2003)。本研究觀察「台農 67 號」不同充實時期穀粒外觀變化之情形，結果由圖 1 顯示「台農 67 號」在不同充實時期，穀粒的重量變化很大。在抽穗初期乾物質累積較為緩慢，14 d 後乾物質快速累積，至 28 d 後乾物質累積逐漸減緩。而不同抽穗時期之強勢粒的重量，也由原本抽穗後 7 d 的 0.027 g 增加至 0.439 g，增加重量高達 16 倍以上。此外，並觀察到在抽穗 28 d 後，穀粒顏色由綠色轉變成金黃色，由此顯示「台農 67 號」再抽穗 28 d 後進入成熟時期。當穀粒成熟時胚乳內乾物質繼續增加，水分已逐漸地減少並由乳漿狀轉變為團狀，且胚乳的中心部分也轉變為透明並向外擴展 (Matsuo & Hoshikawa 1993; Krishnan & Dayanandan 2013; Zhou *et al*. 2013)。

近年來數位化影像處理技術發展迅速，已逐步的使用於農業自動化之研究，並可以協助傳統以肉眼觀察之辨識工作。影像處理分析研究用於小麥最多 (Travis & Draper 1985; Shouche *et al*. 2001)。Paliwal *et al*. (1999) 使用 Canada Western Red Spring wheat, Canada Western Amber Durum wheat, barley, oats, and rye 共 5 種小麥品種為樣本，以小麥的外型輪廓、長度和顏色為參數的基礎，進行分析辨別率可以高達 93%。Paliwal *et al*. (2001, 2003) 使用 5 種小麥品種為樣本，以影像處理技術擷取小麥外觀的面積、周長、最長軸、最短軸等 8 個及 230 個外觀參數，結果可以有效且適合作小麥品種的分類。目前穀粒影像分析技術的使用，可以準確及快速地量測出穀粒的特徵及其形狀參數，據此應用於辨識品種間或品種內粒形的變化。Liu *et al*. (2005) 將 5 種水稻的外觀形狀及顏色作為參數，利用影像處理技術及類神經網路進行品種鑑別，在 20 個參數中可以有效的進行水稻品種的判別分析。本研究利用影像掃描分析「台農 67 號」不同充實時期穀粒外形的變化，試驗的結果指出不同充實時期，穀粒外形變化以內穎變化較外穎明顯，且不同充實階段穀粒厚度會隨著充實時間的增加會有增加的現象。根據 Matsuo & Hoshikawa (1993) 指出水稻開花時子房位於偏內穎一側，受精後子房先是斜向內穎伸長，然後增加穀粒寬度與厚度，本研究試驗結果以內穎變化較外穎明顯此與說明相同。

另外「台農 67 號」不同充實時期，穀粒外形輪廓量測的結果顯示，抽穗後 35 d 最長、最寬且最厚，充實期穀粒長寬比值的變化會隨著充實期時間的增加，比值會有降低的情形。由此可知，穀粒的生長是一種規律化的生長，穀粒生長時先進行長、寬的生長，最後增加厚度。當穀粒之粒長、粒寬及粒厚度的生長定型後，穀粒大小就不再改變；並在成熟後期會因水分含量的降低，穀粒大小會略有變小的情形。

本研究利用影像分析技術進行「台農 67 號」不同充實期穀粒量測，除了可以提供快速非破壞性測量外，其目的是透過取得水稻種子外觀影像，並將外觀特性性狀量化進行分析處理後。未來可進一步開發成水稻種子影像判別資訊系統，使水稻種子檢測工作及純度判別具

備快速檢測、品種判別的功能，並期能達到田間即時判別之目標。同時，更可進一步擴大成為國內水稻育種及品種權認證的背景資料庫。

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Study on Changes of Grain Shape of Rice Cultivar 'TNG67' during Filling Stage by Image Analysis

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Abstract

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A software was developed to feature the grain's shape through digital images taken during grain filling stage in this study. First top 2nd, 3rd, 4th and 5th single grain sampled from top 5 primary branches of each panicle were collected for image analysis in 7, 14, 21, 28, 35, 42 days after heading for rice cultivar 'TNG67'. The results were shown that there are more significant differences on grain shape during filling stage in inner husk than outer husk, and there is no significant difference in outer husk after heading date. Main differences of contour were observed in thickness of grain, and increased dramatically from the 7th day after heading date. There were most significant differences between 105–150 degree of angle in inner husk, and increasing thickness was observed as grains' filling time increased. The high throughput and efficient method was developed for featuring contour of rice grain by image software to evaluate the differences of grain shape during the filling stage.

Key words: Rice, 'TNG67', Grain filling stage, Primary branches, Image analysis.

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台灣農業研究 (J. Taiwan Agric. Res.) 63(2):129–142 (2014)
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台灣進口植物農產品檢出盾介殼蟲 (半翅目：介殼蟲總科：盾介殼蟲科)

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摘要

陳淑佩、翁振宇、吳文哲。2014。台灣進口植物農產品檢出盾介殼蟲 (半翅目：介殼蟲總科：盾介殼蟲科)。台灣農業研究 63(2):129–142。

本文整理 2001–2013 年 6 月間，行政院農業委員會動植物防疫檢疫局各分局，現場檢疫人員後送進口植物農產品檢出之 2,471 筆介殼蟲總科樣品，經由農業試驗所的鑑定結果，其中盾介殼蟲科為 441 筆，分屬於 20 屬 34 種，其中包括 4 屬 4 種台灣未記錄種：箭頭介殼蟲 [*Unaspis yanonensis* (Kuwana)，法定檢疫介殼蟲]、柑桔白輪盾介殼蟲 (*Aulacaspis citri* Chen)、蠣形齒盾介殼蟲 [*Diaspidiotus ostreaeformis* (Curtis)] 及杉刺圓盾介殼蟲 [*Oceanaspidiotus spinosus* (Comstock)]。檢出種類均製成玻片標本並存放於行政院農業委員會農業試驗所應用動物組昆蟲標本館，作為國外進口農產品檢出害蟲之存證標本，其資料可作為日後潛在農業外來種害蟲的風險評估依據。

關鍵詞：盾介殼蟲、進口植物農產品、檢疫害蟲、台灣。

前言

半翅目 (Hemiptera)、介殼蟲總科 (Coccoidea)、盾介殼蟲科 (Diaspididae) 之種類體型微小，常以蟲體或卵隨苗木、接穗、種子、果實、塊莖及鱗莖等農產品傳播 (Chen *et al*. 2011a)，衍生植物檢防疫工作上的問題。迄今全世界已知盾介殼蟲種類超過 2,533 種 (Ben-Dov & Miller 2013)，台灣已記錄 70 屬 236 種 (Takagi 1969, 1970; Tao 1978, 1999; Tao & Wong 1982; Chou & Wong 2000; Jiang 2002, 2004; Ben-Dov & Miller 2013)。

此類昆蟲顯著雌雄異型，營兩性生殖或孤雌生殖，卵生或卵胎生。一年一個世代或多個世代，世代數重疊，其雄成蟲具翅一對，具微弱之飛行能力 (Kosztarab 1990; Wong *et al*. 1999)。第一齡若蟲具足，雌成蟲足退化，固著於寄主植物上以刺吸式口器爲害，嚴重時造成葉片枯黃、花朵枯萎及減弱生長趨勢或植株死亡 (Kosztarab 1990; Wong *et al*. 1999; Chen *et al*. 2011a)。或因取食造成植物組織傷口，可能成爲病原菌侵入的途徑，引發病原菌的感染。除直接危害外，有些種類如梨齒盾介殼蟲 [*Diaspidiotus perniciosus* (Comstock)]，在吸食過程中將含有毒素之唾液注入寄主植物組織，刺激維管束組織增生或變形，影響植物正常生長，造成枝幹黃化及果實被爲害處呈紅暈狀，進而影響商品價值 (Chen *et al*. 2011a)。因此，檢防疫單位必需加強進口植物農產品盾介殼蟲類有害生物之檢出，以降低此類害蟲入侵台灣並危害相關農作物之風險。

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材料與方法

盾介殼蟲科之鑑定樣品來源

行政院農業委員會動植物防疫檢疫局 (Bureau of Animal and Plant Health Inspection and Quarantine, Council of Agriculture; BAPHIQ) 各分局，自 2001–2013 年 6 月間，分別送檢 2,560 筆 (包括含進口植物農產品之實體樣品或 70% 酒精浸泡蟲體標本) 之進口植物農產品樣本 (來自 26 個國家及桃園國際機場回收箱之樣品) 至農業試驗所，經鑑定介殼蟲總科計有 2,471 筆樣本 (Chen *et al*. 2011b)，其中 441 筆樣本屬於盾介殼蟲科。

送檢介殼蟲樣本之影像拍攝及標本處理方法

送檢樣品先進行基本資料登錄，包括送檢單位、輸入國家、農產品中英俗名或學名 (主要參照動植物防疫檢疫局之植物檢疫詞彙中英對照表檢疫篇)、防檢局申請號碼等。此外，對含害蟲之進口農產品實體樣品如果實或植株，再利用實體巨視顯微鏡 (Leica Z16 Apo Stereomicroscope, Switzerland) 並配合自動步進馬達 Piezo 控制調焦器 (Leica Piezo Z-drive, Switzerland)、彩色 RGB CCD 攝影機 (Leica DFC 420 Digital Microscope Cameras, Switzerland)、 電子式標準色溫環形燈組、標準色溫反射台組 (5000 R/L, ring light LED, Switzerland) 拍攝介殼蟲生態及危害狀等，再利用毛筆將欲製成標本之蟲體刷入含 70% 酒精之玻璃容器中，以待製作玻片標本。玻片標本製作流程如下：(1) 以 70% 酒精浸泡標本，並以毛筆將蟲體外殼去除；(2) 將蟲體浸漬於 10% 氫氧化鉀 (KOH) 溶液中 12 h，並分別在蟲體頭胸部附近及腹部 2/3 處，利用微針穿刺體壁，再以微針輕壓蟲體，使其體內內容物藉由孔洞處排出，以達透化；(3) 利用冰醋酸進行中和 1–2 min 後，將蟲體置於洋紅染色，隔夜後再依序以 70、80、90、95 及 100% 酒精各放置 1–2 min，進行脫水處理；(4) 將 Euparal 膠滴於玻片正中央，將蟲體放入膠液內，經整理蟲體姿勢後，覆蓋玻片，以完成封片過程；(5) 最後放入 40–45℃烘箱內 5–7 d，完成玻片標本的製作。

依據盾介殼蟲之專著 (Williams & Watson 1988) 及常用術語 (圖 1)，利用光學顯微鏡 (Leitz DMRB, Leica, Milton Keynes, U.K.) 進行盾介殼蟲玻片形態之種類鑑定。對鑑定介殼蟲的存證標本資料，除鑑定種類外，亦包括異名 [即同種異名 (Synonym)]、鑑定特徵、寄主植物、地理分佈、標本來源 (如美國)、數量 (如 3♀)、採樣日期 (如 03-VI-2010)、攔截單位 (防檢局，以 BAPHIQ 代表) 及檢出 (以 ex 代表) 農產品等資料。

結果

進口台灣之植物農產品檢出盾介殼蟲種類及其來源國家與農產品

從 2001–2013 年 6 月進口植物農產品檢出盾介殼蟲之樣品共 441 筆，占檢出介殼蟲總科 (2,471 筆) 之 17.85%，檢出之盾介殼蟲種類及其檢出國家及進口植物農產品種類列出如表 1。經分析後，以美國進口之農產品被檢出率所佔比率最高，占檢出盾介殼蟲樣品之 27.66%，包括蘋果 (*Malus pumila* Mill.)、酪梨 (*Persea americana* Mill)、月桂葉 (*Laurus nobilis* L.)、油桃 [*Prunus persica* var. *nectarina* (L.)]、葡萄柚 (*Citrus paradisi* Macfad.)、奇異果 (*Actinidia chinensis* Planch.)、檸檬 (*Citrus limon* Burm.)、柳橙 (*Citrus sinensis* Osbeck)、桃子 (*Prunus persica* Stokes)、李子 (*Prunus domestica* L.)、柑橘 (*Citrus* spp.) 等，其中以柳橙 (50 筆)、酪梨 (23 筆) 及葡萄柚 (22 筆) 被檢出含盾介殼蟲比率較高。

泰國進口之農產品被檢出帶有盾介殼蟲率為 25.85%，僅次於美國，檢出之 114 筆植物農產品，包括黑胡椒 (*Piper nigrum* L.)、榴槤 (*Durio zibethinus* Murr.)、山竹 (*Garcinia mangostana* L.)、泰國青檸葉 (*Citrus hystrix* DC.)、紅毛丹 (*Nephelium lappaceum* L.)、假蒟 (*Pi. sarmentosum* Roxb.)、雅囊葉 [*Tiliacora triandra* (Colebr.)]、蘭薩果 (*Lansium domesticum* Correa)、檳榔 (*Areca catechu* L.)、龍眼 (*Euphoria longana* Lam.)、柑橘、蘘荷花 [*Zingiber mioga* (Thunb.)]、檬果 (*Mangifera*

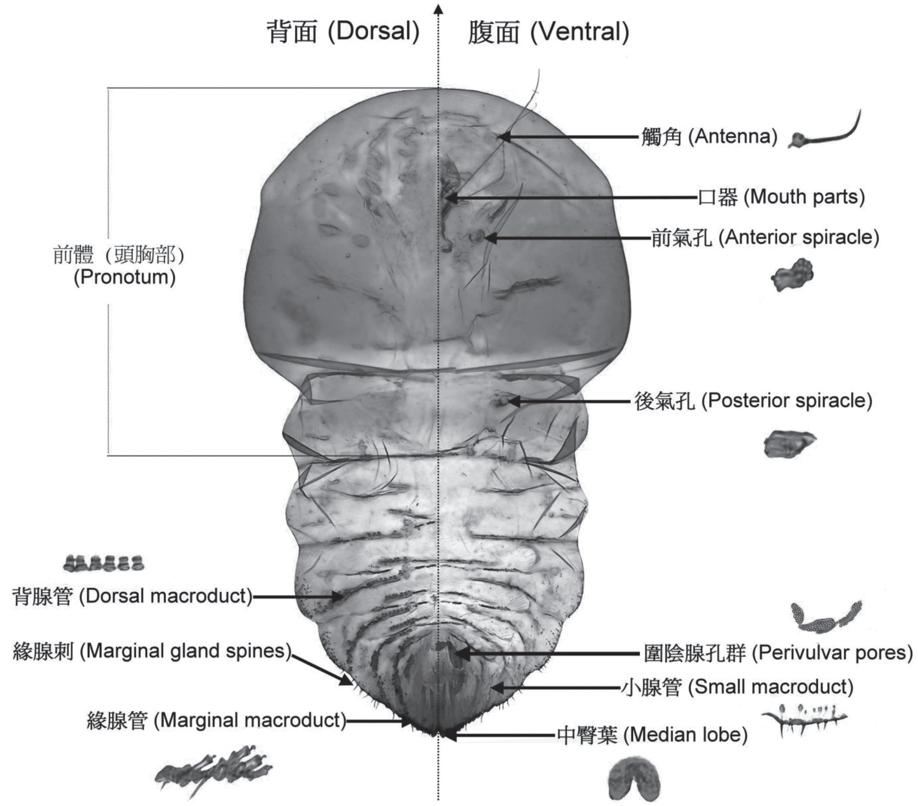

圖 1. 盾介殼蟲雌成蟲形態圖。

Fig. 1. General morphology of an adult female armored scale.

indica L.) 等 13 種農產品，以黑胡椒 (50 筆) 被檢出筆數最多。

印尼進口之農產品占檢出盾介殼蟲筆數比率之 9.75%，計 43 筆居第三位，包括彎木屬 [*Cochlospermum vitifolium* (Willd.)]、山竹及雞蛋花 (*Olumeria* spp.)，其中以山竹被檢出 41 筆爲最大宗 (山竹已因檢疫果實蠅，97 年 6 月在中華民國輸入植物或植物產品檢疫規定之植物防疫檢疫法第十四條第一項規定中禁止輸入)。

日本進口植物農產品檢出含盾介殼蟲共 41 筆，占檢出盾介殼蟲筆數 9.30%，包括蘋果、蜘蛛抱蛋 (*Aspidistra elatior* Blume)、黑松 (*Pinus thunbergii* Parl.)、奇異果、檸檬、羅漢松 [*Podocarpus macrophyllus* (Thunb.)]、桃子、梨 (*Pyrus communis* L.)、李子、柑橘等，其中以柑橘 (12筆)、羅漢松 (8筆) 及桃子 (8筆) 被檢出筆數最多。

越南進口之農產品檢出含盾介殼蟲 25 筆，占檢出盾介殼蟲筆數 5.67%，包括紅毛丹、火龍果 (*Hylocereus* spp.)、薑 (*Zingiber officinale* Roscoe)、檸檬香茅 [*Cymbopogon citratus* (DC.)]，其中以火龍果 (20 筆) 被檢出筆數最多。

中國大陸 (17 筆)、法國 (9 筆) 及義大利 (3 筆) 進口植物農產品被檢出盾介殼蟲樣品雖只占檢出總數 3.85、2.04 及 0.68%，但幾乎全來自奇異果的樣品，各爲 12 筆、9 筆及 3 筆。

南非 (16 筆) 及菲律賓 (10 筆) 進口植物農產品被檢出盾介殼蟲樣品占檢出總數之 3.63% 及 2.27%，主要分別來自柳橙 (10 筆) 及鳳梨 [*Ananas comosus* (L.) Merr.] (9 筆)。

澳大利亞 (12 筆)、智利 (10 筆) 及紐西蘭 (10 筆) 檢出盾介殼蟲樣品數低，分別占檢出

表 1. 2001–2013 年 6 月台灣進口植物農產品檢出盾介殼蟲之國家及農產品種類。

Table 1. The imported countries and categories of plant products with Diaspididae inspected from the quarantine agricultural products to Taiwan from 2001 to June 2013.

Imported country[z]	No. of samples	No. of inspected samples (major plant products)	% inspection (441 samples)
Australia	12	5	2.72
Chile	10	4	2.27
China	17	5	3.85
France	9	1	2.04
Hong Kong	1	1	0.23
Indonesia	43	3	9.75
Italy	3	1	0.68
Japan	41	10	9.30
Korea	2	1	0.45
Malaysia	1	1	0.23
Myanmar	1	1	0.23
New Zealand	10	5	2.27
Singapore	1	1	0.23
South Africa	16	3	3.63
Netherlands	1	1	0.23
Philippines	9	2	2.04
Thailand	114	13	25.85
USA	122	11	27.66
Vietnam	25	3	5.67
Unknown	3	1	0.68

[z] Country names were sorted by alphabetical order.

介殼蟲筆數比率爲 2.72、2.27 及 2.27%，多以柑橘、桃子及李子爲主。

其餘檢出盾介殼蟲的國家爲韓國 (2 筆)、香港、馬來西亞、緬甸、荷蘭、新加坡 (各 1 筆) 及樣品來源無記錄 (3 筆)。

進口農產品檢出盾介殼蟲之種類

檢出盾介殼蟲科 (Diaspididae) 樣本共 441 筆當中，製作後的玻片標本經光學顯微鏡鑑定其種類計 20 屬 34 種。檢出的盾介殼蟲科種類、進口國家和農產品種類如表 2。除柑桔白輪盾介殼蟲 (*Aulacaspis citri* Chen)、蠣形齒盾介殼蟲 [*Diaspidiotus ostreaeformis* (Curtis)]、杉刺圓盾介殼蟲 [*Oceanaspidiotus spinosus* (Comstock)] 及箭頭介殼蟲 [*Unaspis yanonensis* (Kuwana)，法定檢疫介殼蟲] 爲台灣未記錄種外，其餘種類台灣均有記錄，包括擬櫛圓盾介殼蟲 [*Abgrallaspis cyanophylli* (Signoret)]、橘紅腎圓盾介殼蟲 [*Aonidiella aurantii* (Maskell)]、橘黃腎圓盾介殼蟲 [*Aonidiella citrina* (Coquillett)]、凹圓盾介殼蟲 (*Aspidiotus excise* Green)、常春藤圓盾介殼蟲 (*Aspidiotus nerii* Bouche)、褐圓盾介殼蟲 [*Chrysomphalus aonidum* (Linnaeus)]、橙褐圓盾介殼蟲 [*Chrysomphalus dictyospermi* (Morgan)]、梨齒盾介殼蟲 [*Diaspidiotus perniciosus* (Comstock)]、鳳梨盾介殼蟲 [*Diaspis bromeliae* (Kerner)]、仙人掌盾介殼蟲 [*Diaspis echinocacti* (Bouche)]、竹禾盾介殼蟲 [*Duplachionaspis divergens* (Green)]、圍盾介殼蟲 [*Fiorinia fioriniae* (Targioni Tozzetti)]、杉柏圍盾介殼蟲 (*Fiorinia japonica* Kuwana)、椰子櫛圓盾介殼蟲 [*Hemiberlesia lataniae* (Signoret)]、棕櫛圓盾介殼蟲 [*Hemiberlesia rapax*

表 2. 2001 至 2013 年 6 月檢出盾介殼蟲雌成蟲種類、植物農產品種類與其輸出國。

Table 2. The imported countries and categroies of plant products with Diaspididae female adults from the quarantine agricultural products to Taiwan from 2001 to June 2013.

Species [z, y]	Number of samples with Diaspididae female adults	No. of plant products (scientific name)	Number of imported countries inspected (Diaspididae female adults)
Aonidiella aurantii	63	10 (Unknown, *Citrus bergamia*, *Citrus hystrix*, *Citrus limon*, *Citrus paradisi*, *Citrus sinensis*[x], *Citrus* spp., *Laurus nobilis*, *Podocarpus macrophyllus*, *Tiliacora triandra*)	8 (Australia, China, Japan, the Netherlands, South Africa, Thailand, Unknown, USA)
Pseudaulacaspis cockerelli	44	9 (Unknown, *Actinidia chinensis*, *Areca cathechn*, *Diospyros kaki*, *Garcinia mangostana*[x], *Mangifera indica*, *Nephelium lappaceum*, *Piper nigrum*, *Piper sarmentosum*)	5 (China, Indonesia, Korea, Thailand, Unknown)
Lepidosaphes beckii	35	4 (*Citrus paradisi*, *Citrus sinensis*[x], *Citrus* spp., *Piper betle*, *Citrus paradisi*)	4 (China, Myanmar, South Africa, USA)
Diaspidiotus perniciosus	28	9 (*Actinidia arguta*, *Actinidia chinensis*, *Citrus* spp., *Malus pumila*, *Nephelium lappaceum*, *Persea americana*, *Prunus domestica*, *Prunus persica*, *Prunus persica* var. *nectarina*)	5 (Chile, Japan, New Zealand, USA, Vietnam)
Pinnaspis aspidistrae	26	4 (*Citrus limon*, *Citrus paradisi*, *Citrus sinensis*, *Piper nigrum*[x])	3 (Japan, Thailand, USA)
Hemiberlesia lataniae	26	6 (*Actinidia chinensis*, *Citrus paradisi*, *Persea americana*[x], *Prunus persica*, *Prunus persica* var. *nectarina*, *Vaccinium* spp.)	4 (Australia, Japan, New Zealand, USA)
Pseudaulacaspis pentagona	25	4 (*Actinidia chinensis*[x], *Olumeria* spp., *Prunus persica*, *Pyrus communis*)	6 (China, France, Indonesia, Italy, Japan, USA)
Diaspis echinocacti	21	1 (*Hylocereus* spp.[x])	2 (Malaysia, Vietnam)
Parlatoria pergandii	19	4 (*Citrus hystrix*, *Citrus paradisi*, *Citrus sinensis*[x], *Citrus* spp.)	2 (Thailand, USA)
Chrysomphalus dictyospermi	18	1 (*Garcinia mangostana*[x])	2 (Indonesia, Thailand)
Parlatoria ziziphi	9	1 (*Citrus hystrix*[x])	1 (Thailand)
Unaspis yanonensis[w]	9	1 (*Citrus* spp.[x])	1 (Japan)
Diaspis bromeliae	6	1 (*Ananas comosus*[x])	1 (the Philippines)
Hemiberlesia rapax	5	5 (*Actinidia chinensis*, *Citrus sinensis*, *Citrus* spp., *Persea americana*, *Punica granatum*)	4 (China, Japan, New Zealand, USA)
Lepidosaphes gloverii	4	1 (*Citrus paradisi*)	1 (USA)
Chrysomphalus aonidum	3	1 (*Garcinia mangostana*)	2 (China, Thailand)
Pinnaspis uniloba	3	1 (*Piper nigrum*)	1 (Thailand)
Aonidiella citrina	2	2 (*Podocarpus macrophyllus*, *Tiliacora triandra*)	2 (Japan, Thailand)
Aspidiotus nerii	2	2 (*Actinidia chinensis*, *Protea cynaroides*)	2 (South Africa, USA)
Diaspidiotus ostreaeformis[w]	2	2 (*Malus pumila*, *Prunus persica*)	2 (New Zealand, USA)
Lepidosaphes pini	2	1 (*Pinus thunbergii*)	1 (Japan)
Melanaspis smilacis	2	1 (*Ananas comosus*)	1 (Philippines)
Abgrallaspis cyanophylli	1	1 (*Garcinia mangostana*)	1 (Thailand)

表 2. 2001 至 2013 年 6 月檢出盾介殼蟲雌成蟲種類、植物農產品種類與其輸出國 (續)。

Table 2. The imported countries and categroies of plant products with Diaspididae female adults from the quarantine agricultural products to Taiwan from 2001 to June 2013. (continued)

Species [z, y]	Number of samples with Diaspididae female adults	No. of plant products (scientific name)	Number of imported countries inspected (Diaspididae female adults)
Aulacaspis citri[w]	1	1 (*Cymbopogon citratus*)	1 (Vietnam)
Duplachionaspis divergens	1	1 (*Cymbopogon citratus*)	1 (Vietnam)
Fiorinia fioriniae	1	1 (*Citrus hystrix*)	1 (Thailand)
Fiorinia japonica	1	1 (*Podocarpus macrophyllus*)	1 (Japan)
Lepidosaphes conchiformis	1	1 (*Pyrus communis*)	1 (Japan)
Lepidosaphes leei	1	1 (*Podocarpus macrophyllus*)	1 (Japan)
Lepidosaphes ulmi	1	1 (*Malus pumila*)	1 (Chile)
Lopholeucaspis japonica	1	1 (*Malus pumila*)	1 (Japan)
Oceanaspidiotus spinosus[w]	1	1 (*Zingiber mioga*)	1 (Thailand)
Aspidiotus excises	1	1 (*Garcinia mangostana*)	1 (China)
Thysanofiorinia nephelii	1	1 (*Euphoria longana*)	1 (Thailand)

[z] Family names arranged by the number of female adult Diaspididae among the 366 cases;
[y x]The main agricultural products were inspected; [w]The species have not been recorded in Taiwan. (y and x need to be well defined separately.)

(Comstock)]、橘紫蠣盾介殼蟲 [*Lepidosaphes beckii* (Newman)]、梨蠣盾介殼蟲 [*Lepidosaphes conchiformis* (Gmelin)]、橘長蠣盾介殼蟲 [*Lepidosaphes gloverii* (Packard)]、冬青蠣盾介殼蟲 (*Lepidosaphes leei* Takagi)、松蠣盾介殼蟲 [*Lepidosaphes pini* (Maskell)]、日本長片盾介殼蟲 [*Lopholeucaspis japonica* (Cockerell)]、榆蠣盾介殼蟲 [*Lepidosaphes ulmi* (Linnaeus)]、菝契黑圓盾介殼蟲 [*Melanaspis smilacis* (Comstock)]、糠片盾介殼蟲 (*Parlatoria pergandii* Comstock)、黑片盾介殼蟲 [*Parlatoria ziziphi* (Lucas)]、柑橘并盾介殼蟲 [*Pinnaspis aspidistrae* (Signoret)]、合葉并盾介殼蟲 [*Pinnaspis uniloba* (Kuwana)]、椰子擬輪盾介殼蟲 [*Pseudaulacaspis cockerelli* (Cooley)]、桑擬輪盾介殼蟲 [*Pseudaulacaspis pentagona* (Targioni-Tozzetti)] 及纓圍盾介殼蟲 [*Thysanofiorinia nephelii* (Maskell)]。部分因蟲體太小，或發育初期，未達可鑑定齡期，僅能鑑定至屬或科的若蟲，其進口國家和農產品種類如表 3。

送檢樣品中以雌成蟲鑑定出種類之盾介殼蟲集中於 10 種，分別為橘紅腎圓盾介殼蟲 (63 筆)、椰子擬輪盾介殼蟲 (44 筆)、橘紫蠣盾介殼蟲 (35 筆)、梨齒盾介殼蟲 (28 筆)、柑桔并盾介殼蟲 (26 筆)、椰子櫛圓盾介殼蟲 (26 筆)、桑擬輪盾介殼蟲 (25 筆)、仙人掌盾介殼蟲 (21 筆)、糠片盾介殼蟲 (19 筆)、橙褐圓盾介殼蟲 (18 筆)，共計 305 筆，占可鑑定之雌成蟲檢出筆數 (441 筆) 的 69.16%。而檢出最多筆的橘紅腎圓盾介殼蟲，主要由芸香科果品檢出，僅少數由月桂葉、羅漢松及雅囊葉檢出；檢出次多的椰子擬輪盾介殼蟲，主要來自山竹等 8 種農產品，大多由東南亞進口；檢出的橘紫蠣盾介殼蟲，主要由芸香科檢出；檢出的梨齒盾介殼蟲，由 9 種農產品 (主要桃子及李子) 檢出。柑桔并盾介殼蟲主要從東南亞進口之農產品黑胡椒果實上檢出 21 筆；椰子櫛圓盾介殼蟲

表 3. 2001 年至 2013 年 6 月檢出盾介殼蟲若蟲與其輸出國植物農產品。

Table 3. The genus and inspected frequency of nymphal Diaspididae from the quarantine agricultural products to Taiwan from 2001 to June 2013.

Genera[z]	Number of samples with nymphal Diaspididae	No. of plant products (scientific name)	Number of imported countries inspected nymphal Diaspididae
Pinnaspis	4	2 (*Cochlospermum vitifolium*, *Piper nigrum*)	2 (Indonesia, Thailand)
Pseudaulacaspis	4	3 (*Actinidia chinensis*, *Cymbopogon citratus*, *Podocarpus macrophyllus*)	4 (China, Japan, New Zealand, Vietnam)
Aonidiella	3	3 (*Citrus hystrix*, *Garcinia mangostana*, *Podocarpus macrophyllus*)	2 (Japan, Thailand)
Aulacaspis.	3	3 (*Garcinia mangostana*, *Lansium domesticum*, *Nephelium lappaceum*)	3 (Indonesia, Thailand, Vietnam)
Hemiberlesia	3	2 (*Prunus domestica*, *Prunus persica* var. *nectarina*)	1 (USA)
Aspidiotus	2	2 (*Citrus sinensis*, *Piper nigrum*)	2 (Thailand, USA)
Chionaspis	1	1 (*Leucadendron* spp.)	1 (Australia)
Chrysomphalus	1	1 (*Pinus thunbergii*)	1 (Japan)
Diaspidiotus	1	1 (*Malus pumila*)	1 (Chile)
Lepidosaphes	1	1 (*Pinus thunbergii*)	1 (Japan)
Lopholeucaspis	1	1 (*Piper nigrum*)	1 (Thailand)
Parlatoria	1	1 (*Durio zibethinus*)	1 (Thailand)
Diaspididae	50	12 (*Actinidia chinensis*, *Aspidistra elatior*, *Citrus hystrix*, *Laurus nobilis*, *Mangifera indica*, *Nephelium lappaceum*, *Persea americana*, *Piper nigrum*, *Piper sarmentosum*, *Podocarpus macrophyllus*, *Prunus persica*, *Tiliacora triandra*)	6 (Hong Kong, Japan, the Philippines, Singapore, Thailand, USA)

[z] Genus names arranged by the number of Diaspididae (nymph) among the 75 cases.

要由酪梨果實上檢出 19 筆；桑擬輪盾介殼蟲從 4 種進口植物農產品檢出，其中 21 筆檢出主要由奇異果檢出，仙人掌盾介殼蟲主要從東南亞進口之農產品火龍果檢出 21 筆，糠片盾介殼蟲全部由芸香科果品上檢出 19 筆，橙褐圓盾介殼蟲主要從泰國進口之農產品山竹檢出 18 筆。

零星檢出的盾介殼蟲，包括黑片盾介殼蟲、箭頭介殼蟲 (台灣未記錄)、鳳梨盾介殼蟲、棕櫚圓盾介殼蟲、橘長蠣盾介殼蟲、褐圓盾介殼蟲、合葉并盾介殼蟲、橘黃腎圓盾介殼蟲、常春藤圓盾介殼蟲、蠣形齒盾介殼蟲 (台灣未記錄)、松蠣盾介殼蟲、菝契黑圓盾介殼蟲、擬櫛圓盾介殼蟲、柑桔白輪盾介殼蟲 (台灣未記錄)、竹禾盾介殼蟲、圍盾介殼蟲、杉柏圍盾介殼蟲、梨蠣盾介殼蟲、冬青蠣盾介殼蟲、榆蠣盾介殼蟲、日本長片盾介殼蟲、杉刺圓盾介殼蟲 (台灣未記錄)、凹圓盾介殼蟲及纓圍盾介殼蟲。

綜合上述，2001–2013 年 6 月防檢局檢出，送鑑定之進口植物農產品中，盾介殼蟲樣品 441 筆雖僅占檢出介殼蟲總科 (2,471 筆) 之 17.85%，但檢出的種類共計 20 屬 34 種，其中 4 屬 4 種爲台灣未記錄種類 (雌成蟲)，其描述如下：

柑桔白輪盾介殼蟲 (*Aulacaspis citri* Chen 1954)

鑑定特徵 (圖 2A–C)：雌介殼白色或灰黃色，呈圓形或近圓形，薄而微微隆起，蛻皮接近蟲體中心。雄介殼爲白色，蠟質狀，狹長，兩側平行，背面有 3 條明顯的縱脊線，蛻皮位於前端，淡黃色。雌成蟲體略呈半圓形，體長約 1.5 mm，寬約 0.85 mm；觸角呈小突起，其上具 1 根剛毛 (Chen

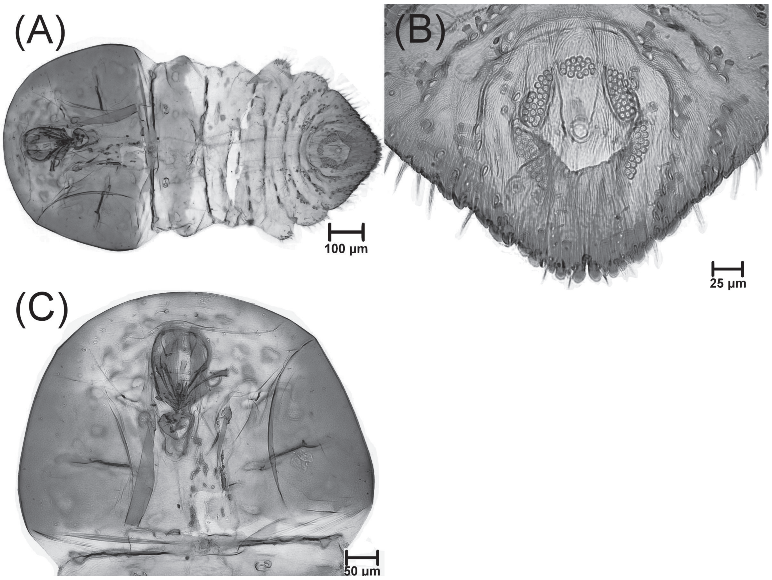

圖 2.　柑桔白輪盾介殼蟲 (*Aulacaspis citri* Chen 1954)。(A) 形態圖；(B) 臀部形態圖；(C) 頭部形態特徵圖。

Fig. 2.　*Aulacaspis citri* Chen, 1954. (A) Slide mounted female, (B) posterior portion of slide mounted female, and (C) anterior portion of slide mounted female.

1954)；管腺發達，呈短條形；臀板具 3 對臀葉 (Pygidial lobes)，中臀葉及第 2 臀葉發達，第 2 臀葉外緣稍呈圓形，其長度短於臀板邊緣的臀棘 (Pygidial plate)；第 3 臀葉呈片狀；肛門比中臀葉小，圍陰戶孔在肛門周圍位置，臀部具 5 群圍陰戶孔 (Perivulvar disc pores)。與台灣存在的牛奶子輪盾介殼蟲 [*Aulacaspis crawii* (Cockerell 1899)] 在形態上相似，常造成種類誤判，可由中臀葉形態、5 群圍陰戶孔及管腺數量 (牛奶子輪盾介殼蟲 5 群圍陰戶孔及管腺之數量皆比柑桔白輪盾介殼蟲多) 加以區分。

寄主植物：木藍、四季橘、柑橘屬、柚、紅柑、甜橙、萊姆、菝葜、楝、樟樹及檸檬等 (Ben-Dov & Miller 2013)。

地理分佈：中國 (Ben-Dov & Miller 2013)。

標本來源：越南，1 ♀，03-VI-2010，BAPHIQ ex 檸檬香茅。

蠣形齒盾介殼蟲 [*Diaspidiotus ostreaeformis* (Curtis 1843)]

異名：*Aspidiotus ostreaeformis*, *Aspidiotus betulae*, *Aspidiotus hippocastani*, *Aspidiotus oxyacanthae*, *Aspidiotus tiliae*, *Diaspis ostreaeformis*, *Diaspis ostraeformis*, *Aspidiotus* (*Diaspidiotus*) *betulae*, *Aspidiotus* (*Evaspidiotus*) *betulae*, *Aspidiotus ostreaeformis oblongus*, *Aspidiotus ostreaeformis magnus*, *Aspidiotus* (*Evaspidiotus*) *ostreaeformis*, *Aspidiotus alma-atensis*, *Aspidiotus* (*Quadraspidiotus*) *ostreaeformis*, *Aspidiotus magnus*, *Aspidiotus oblongus*, *Diaspidiotus alma-atensis*, *Quadraspidiotus alma-atensis*, *Quadraspidiotus williamsi*, *Quadraspidiotus almaatensis*

鑑定特徵 (圖 3A–D)：雌介殼呈圓形，微微隆起；顏色多變化，由黃白色到灰褐色，其介殼直徑 1.4–1.9 mm。雄介殼呈卵形，比雌介殼小，顏色相似於雌介殼，介殼長度 1.0–1.5 mm (Watson 2005)。由玻片標本觀察，雌介殼蟲體呈圓形，觸角呈小突起，其上具 1 根剛毛；管腺發達，呈短條形；臀板具 3 對臀葉，中臀葉及第 2 臀葉發達，第 2 臀葉外緣稍呈鋸齒狀缺刻，其

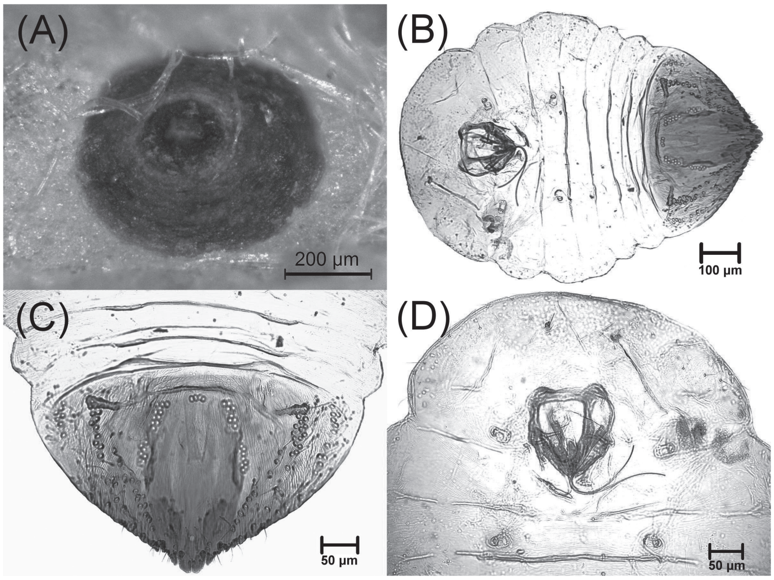

圖 3. 蠣形齒盾介殼蟲 [*Diaspidiotus ostreaeformis* (Curtis 1843)]。(A) 生態圖；(B) 形態圖；(C) 臀部形態圖；(D) 頭部形態特徵圖。

Fig. 3. *Diaspidiotus ostreaeformis* (Curtis 1843). (A) Adult female in life, (B) slide mounted female, (C) posterior portion of slide mounted female, and (D) anterior portion of slide mounted female.

長度短於臀板邊緣的臀棘；第 3 臀葉退化成點狀突起；肛門比中臀葉小，陰戶孔在肛門上方位置，臀部具 5 群圍陰戶孔。

寄主植物：七葉樹屬、千金榆屬、水青岡屬、冷杉屬、皂莢屬、法國梧桐、花楸屬、金雀花屬、柳屬、紅醋栗、核桃、海棗屬、茶藨子屬、梅屬、梨屬、榜屬、無花果、楊梅屬、楊屬、楓樹屬、榆屬、鼠李屬、鉆天楊、榛屬、銀白楊、齊墩果、椴木屬、歐洲石楠、樺木屬、錦雞兒屬、櫟屬、蘋果屬及榅桲屬等 (Ben-Dov & Miller 2013)。

地理分佈：土耳其、中國、巴基斯坦、日本、以色列、加拿大、北韓、尼泊爾、立陶宛、伊拉克、伊朗、匈牙利、印度、吉爾吉斯、西班牙、克羅埃西亞、希臘、亞塞拜然、波蘭、法國、阿扎爾共和國、阿根廷、保加利亞、俄羅斯、哈薩克、科西嘉島、美國、英國、烏茲別克、紐西蘭、捷克、荷蘭、喬治亞、斯洛伐克、斯洛維尼亞、塔吉克、愛爾蘭、瑞士、瑞典、義大利、葡萄牙、德國、摩洛哥、黎巴嫩、澳洲及羅馬尼亞 (Ben-Dov & Miller 2013)。

標本來源：紐西蘭，4♀，12-VIII-2010，BAPHIQ ex 蘋果；美國，1♀，12-VIII-2010，BAPHIQ ex 桃。

杉刺圓盾介殼蟲 [*Oceanaspidiotus spinosus* (Comstock 1883)]

異名：*Aspidiotus spinous*, *Aspidiotus* (*Aspidiotus*) *spinosus*, *Aspidiotus* (*Evaspidiotus*) *spinosus*, *Aspidiotus persearum*, *Aspidiotus* (*Evaspidiotus*) *persearum*, *Acanthaspidiotus borchsenii*, *Acanthaspidiotus spinos*

鑑定特徵 (圖 4A–D)：雌介殼直徑 1.0–1.5 mm，呈微隆起的褐色圓形介殼，其介殼常在植株表皮下，與組織 (如樹皮或種皮) 相混。介殼下的雌成蟲體呈規則的橙黃色梨形，頭胸部和前面腹節的側表皮加厚；氣孔前微微加厚；胸瘤退化呈一色素區，但很少突出；臀前腹節各節每側有一背腺管，臀板褐色；觸角呈瘤狀突起物，其上具 1 根剛毛；管腺發達，呈短條形；臀板

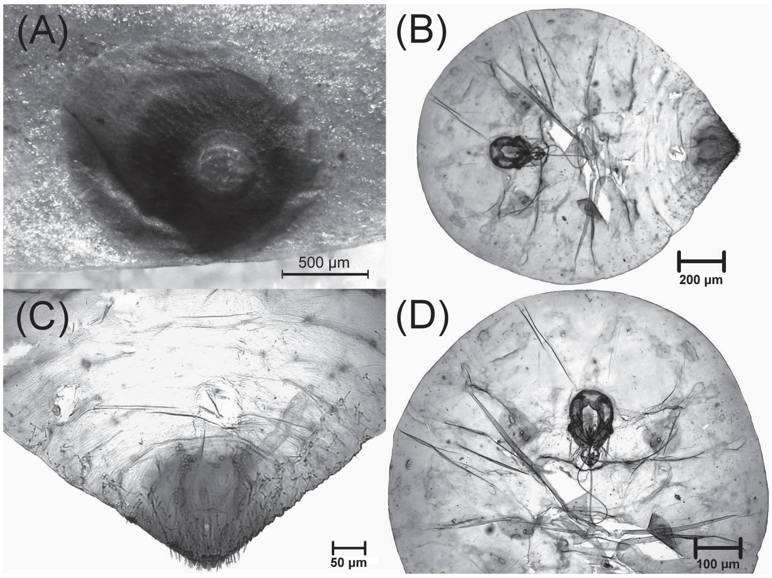

圖 4.　杉刺圓盾介殼蟲 [*Oceanaspidiotus spinosus* (Comstock 1883)]。(A) 生態圖；(B) 形態圖；(C) 臀部形態圖；(D) 頭部形態特徵圖。

Fig. 4.　*Oceanaspidiotus spinosus* (Comstock 1883). (A) Adult female in life, (B) slide mounted female, (C) posterior portion of slide mounted female, and (D) anterior portion of slide mounted female.

具 3 對臀葉及臀棘，中臀葉很發達，長過於寬，對稱而平行，端部呈圓形而兩側具骨化程度明顯的缺刻；中臀葉腹面具加厚的皮層，向內延伸至圍陰腺孔的後緣；第 2 臀葉很小，端部圓形，僅外緣有缺刻；第 3 臀葉退化，呈一三角形的齒狀物，個別個體可能消失，臀棘在背面第 2–3 臀葉基部間出現 (Takagi 1984)；肛門倒梨圓形、近臀部中心部位。陰戶孔在肛門上方位置，臀部具 4 群圍陰戶孔。

寄主植物：山桂花屬、天門冬屬、日本山茶、日本黃楊、月桂、木蘭屬、凹葉柃木、北美木蘭、地中海莢迷、地笋屬、羊蹄甲屬、法國梧桐、長葉繡球、非洲蘇鐵屬、指甲花、柑橘屬、洋玉蘭、洋桐木屬、砂糖椰子、紅豆杉屬、美國波羅花、苧麻屬、茄、茶梅、茶屬、酒瓶蘭、假菝契、假葉樹屬、莢蒾屬、棕竹屬、棕櫚、無花果、番荔枝屬、菝葜屬、楨楠屬、葡萄、葡萄屬、酪梨、榕屬、蒲葵、鳳梨、樟屬、潺槁樹、衛矛屬、錫蘭樟、錦熟黃楊、龍眼、薔薇屬、螺旋長春藤、檬果、繡球花、羅漢松屬、懸鉤子屬及檫樹屬等 (Ben-Dov & Miller 2013)。

地理分佈：熱帶及溫帶地區，包括土耳其、小笠原群島、中國、巴西、巴哈馬、日本、以色列、加那利群島、古巴、尼泊爾、伊朗、印度、多明尼加、百慕達、西西里、西班牙、亞速爾群島、坦尙尼亞、波多黎各、阿扎爾共和國、阿根廷、阿爾及利亞、南非、美國、英國、哥倫比亞、哥斯大黎加、埃及阿拉伯、夏威夷、庫克群島、海地、烏拉圭、秘魯、馬達加斯加、馬德拉群島、密克羅尼西亞聯邦、敘利亞、莫三比克、喬治亞、義大利、葛摩聯邦、葡萄牙、維開斯島、摩洛哥及墨西哥 (Ben-Dov & Miller 2013)。

標本來源：泰國，8 ♀，19-III-2012，BAPHIQ ex 薑荷花。

箭頭介殼蟲 [*Unaspis yanonensis* (Kuwana 1907)]

異名：*Chionaspis citri, Prontaspis yanonensis*

鑑定特徵 (圖 5A–5D)**：**雌介殼呈箭頭形，前狹後寬。長約 2.5–3.5 mm，寬約 1.5–1.9

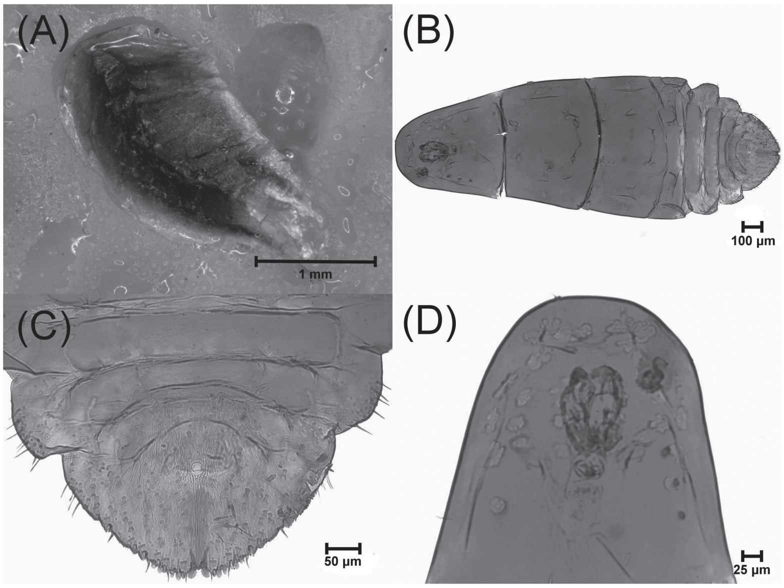

圖 5. 箭頭介殼蟲 [*Unaspis yanonensis* (Kuwana 1907)]。(A) 生態圖；(B) 形態圖；(C) 臀部形態圖；(D) 頭部形態特徵圖。

Fig. 5. *Unaspis yanonensis* (Kuwana 1907). (A) Adult female in life, (B) slide mounted female, (C) posterior portion of slide mounted female, and (D) anterior portion of slide mounted female.

mm，扁平，深褐色或黑褐色，邊緣灰白色，背面中央有一縱脊，兩側有多條淺橫脊線，若蟲蛻皮殼突出在前端，黃褐色；雄介殼呈長條形，兩側平行，長約 1.3–1.6 mm，背面有 3 條縱脊，白色，蠟質。若蟲蛻皮殼貼附在前端；淡黃色透明。雌成蟲體呈茄形，頭、胸部與腹部分界明顯，腹部末端二節側面突出。體橙黃色，後期轉為淡棕色。頭前端呈圓弧狀，呈瘤狀突起物之觸角靠近頭前緣，其上具 1 根剛毛；管腺發達，呈短條形；前氣門具 10–14 個盤孔，後氣門具 2–5 個盤孔、但有時消失；後胸及第一腹節側緣具十餘個錐狀線瘤。臀部圓弧狀，基部具 5–8 根腺刺，端部稍凹入，每側各具緣腺管 7 個、與呈不規則縱列分布之背腺管 50–80 個；臀板具 3 對臀葉及臀棘，中臀葉發達，其左右臀葉呈八字分開而葉緣呈鋸齒狀；第 2 臀葉分成 2 小葉，端部圓形，稍小；第 3 臀葉與第 2 臀葉相似，但較短小；肛門圓形、近臀部中心部位。陰戶孔與肛門相同位置，無圍陰戶孔。本種與桔矢尖介殼蟲 [*Unaspis citri* (Comstock)] 相似，在柑桔類 (*Citrus* spp.) 上亦常混生危害。後者雌成蟲介殼稍小，亦常呈 S 形彎曲，介殼稍隆起。雌成蟲體頭側有一小突起，胸部分節不明顯 (Wong *et al*. 2000)。

寄主植物：山茶、月橘、白蠟樹、佛手柑、吳茱萸、芍藥、柚、枸櫞、枳殼、桂花、茶、甜橙、連翹、番石榴、黃皮、廣東檸檬及龍眼等 (Ben-Dov & Miller 2013)。

地理分佈：中國、巴基斯坦、日本、印尼、印度、亞美尼亞、法國、南韓、香港、泰國、琉球群島、馬來西亞、斐濟、菲律賓、越南、義大利、緬甸及澳洲 (Ben-Dov & Miller 2013)。

標本來源：日本，2♀，25-IV-2013，BAPHIQ ex 柑橘；泰國，2♀，06-IV-2010，BAPHIQ ex 柑橘；日本，1♀，25-XI-2009，BAPHIQ ex 柑橘；日本，6♀，06-XI-2008，BAPHIQ ex 柑橘；日本，4♀，07-II-2007，BAPHIQ ex 柑橘；日本，1♀，04-I-2007，BAPHIQ ex 柑橘；日本，5♀，09-XII-2006，BAPHIQ ex

柑橘；日本，1♀，18-I-2006，BAPHIQ ex 柑橘；日本，2♀，06-XII-2004，BAPHIQ ex 柑橘。

討論

從 2001–2013 年 6 月進口植物農產品檢出盾介殼蟲之樣品共 441 筆，雖然僅占檢出介殼蟲總科 (2,471 筆) 之 17.85%，但檢出的種類高達 20 屬 34 種，其中 18 屬 30 種爲台灣已記錄的種類，4 屬 4 種爲台灣未記錄種類。由此顯示，此類害蟲易隨植物農產品入侵台灣。送檢樣品經分析後，以美國及泰國大宗進口之農產品被檢出率所佔比率最高，共占檢出盾介殼蟲樣品之 53.51%，自美國農產品檢出 1 種台灣未記錄種，泰國檢出 2 種台灣未記錄種，其他檢出種類爲已記錄種類。檢出未記錄盾介殼蟲種類對台灣農產品的風險，以柑桔白輪盾介殼蟲爲例，此害蟲寄主多以柑橘科果樹爲主，一年具 5 世代，其地理分佈僅記錄分佈於中國 (Ben-Dov & Miller 2013)。本研究在越南進口之檸檬香茅檢出之柑桔白輪盾介殼蟲爲世界首次紀錄，檸檬香茅上已記錄的介殼蟲種類僅有工背硬介殼蟲 [*Saissetia oleae* (Olivier) 及 *Toumeyella cubensis* Heidel & Ko (Ben-Dov & Miller 2013)]。若此害蟲不慎由農產品輸入而建立族群，將衝擊柑橘果樹之產業。此外，蘋果及桃子是輸入植物農產品之大宗，本文檢出之蠣形齒盾介殼蟲主要棲息及爲害寄主果樹的莖部及樹幹，嚴重時可導致寄主植物組織乾枯 (Watson 2005)。此害蟲亦可在果實上發生，並造成果品上具紅色斑點之爲害狀 (Watson 2005)。此檢測結果對於日後進行輸入果品的風險評估時，可提供相關資訊，以利合理進行蠣形齒盾介殼蟲的風險評估。又薑荷花已記錄的介殼蟲種類僅有 [*Drosicha corpulenta* (Kuwana)] (Ben-Dov & Miller 2013)，而本研究在薑荷花檢出之杉刺圓盾介殼蟲爲世界首次紀錄。因其寄主亦包含球根花卉，故日後從泰國引入天南星科的植物時，需特別小心有無此害蟲在球莖部表面；多次從日本和泰國的柑橘檢出的箭頭介殼蟲爲台灣列名之檢疫害蟲，在日本及中國等地一年有 2–4 世代。由於可嚴重爲害柑橘類果樹而爲爲世界重要的害蟲 (Ben-Dov & Miller 2013)，且皆由柑橘類果品檢出，故日後對日本輸入的柑橘類果品檢測時，值得注意是否有此害蟲存在。上述的資料已建置在植物檢疫有害生物資訊系統 (http://DigiPest.tari.gov.tw/)，以供線上快速查詢其害蟲鑑定，以達成爲我國農產品降低被隨農產品而進入之檢疫害蟲爲害的風險，進而提升我國之貿易競爭力。

此外，在送檢樣品中，部分送檢樣品以卵或是若蟲形式被檢測出，故未來應以已鑑定的成蟲形態爲基石，進一步開發檢出之卵與若蟲分子生物鑑定技術爲輔助，建立形態與分生雙重鑑定的資訊，以利檢疫工作達快速正確之目標。

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Armored Scale Insects (Hemiptera: Coccoidea: Diaspididae) Intercepted from Imported Plant Products to Taiwan

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Abstract

Chen, S. P., J. Y. Wong, and W. J. Wu. 2014. Armored scale insects (Hemiptera: Coccoidea: Diaspididae) intercepted from imported plant products to Taiwan. J. Taiwan Agric. Res. 63(2):129–142.

Since 2001, the scale insects inspected from the imported agricultural products by Bureau of Animal and Plant Health Inspection and Quarantine (BAPHIQ), Council of Agriculture, have been sent to Taiwan Agricultural Research Institute (TARI) for identification. A total of 2,471 scale insect samples were inspected from 2001 to June 2013, of which 441 samples belonging to 34 species of 20 genera of Diaspididae (Hemiptera: Sternorrhyncha: Coccoidea) were identified. Among them, 4 species of 4 genera have not been recorded in Taiwan before, including *Unaspis yanonensis* (Kuwana), *Aulacaspis citri* Chen, *Diaspidiotus ostreaeformis* (Curtis) and *Oceanaspidiotus spinosus* (Comstock). The information could be used as a basis for the risk assessment of exotic species becoming potential pest in Taiwan.

Key words: Quarantine pest, Coccoidea, Imported agricultural products, Taiwan.

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台灣農業研究 (J. Taiwan Agric. Res.) 63(2):143–150 (2014)
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Pseudomonas cichorii 引起之番茄細菌性葉斑病

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摘要

蔡佳欣、安寶貞、呂昀陞、陳美德、黃淑苓、彭玉花。2014。*Pseudomonas cichorii* 引起之番茄細菌性葉斑病。台灣農業研究 63(2):143–150。

南投縣與花蓮縣部分番茄栽植田於 2010–2011 年期間，發現植株葉片出現墨綠至暗褐色之不規則形或圓形壞疽之水浸狀斑點病徵，經由病組織分離出一種病原細菌，該細菌可誘導萬國士煙草產生過敏性反應。繼將該細菌接種至番茄葉片，可產生相同病徵，完成柯霍氏法則測試。此病原細菌經生理生化測試、Biolog 鑑定系統分析、16S rDNA 序列比對，並以專一性引子對 SfL1/SfR2 進行 PCR 增幅鑑定，確認此病原細菌為 *Pseudomonas cichorii*。此為 *P. cichorii* 感染番茄葉片在台灣的首次報導。以濾紙圓盤擴散法測試 10 種市售藥劑對該病原菌的抑制圈大小，結果顯示以鏈四環黴素之抑制效果最佳。

關鍵詞：番茄、細菌性葉斑病、細菌性葉斑病菌、藥劑篩選。

前言

番茄 (*Solanum lycopersicum* L.) 屬於茄科 (Solanaceae) 茄屬 (Solanum)，是世界性的重要蔬菜之一，品種繁多。番茄於台灣栽種面積達 4,500 ha，每公頃收穫量約為 24,750 kg，其中以嘉義縣種植面積最大 (Anonymous 2013)。在台灣有頗多危害番茄的病害，包括疫病、晚疫病、早疫病、萎凋病、白粉病、青枯病、細菌性軟腐病、細菌性斑點病及病毒病等 (Hsu *et al*. 2002)。自 2010 年起南投縣與花蓮縣地區番茄栽植田，部分田區的植株於定植 1–2 個月後之生長期，葉片出現不尋常的病徵，病株葉片出現墨綠至暗褐色之不規則形或圓形之水浸狀斑點病徵，葉片病害嚴重田區之發病比率達 30% 以上。其墨綠色之水浸狀病斑與常見之細菌性斑點病徵有所差異，嚴重發病株整葉褐化壞疽。由病葉切取病斑於光學顯微鏡下鏡檢，可見細菌自切口大量湧出，推測可能為細菌性所引起結果。

由於此一病害已分別發現於不同縣市，為避免造成大量經濟損失，本研究主要目的即在對此病之病因及病原菌特性進行探討，同時於室內篩選合適防治藥劑，以供番茄栽培及病害防治參考。

材料與方法

菌株來源

本試驗於南投縣與花蓮縣之番茄發病田區採集具墨綠至暗褐色病斑之葉片 (圖 1)，切取葉部組織後以 1% 次氯酸鈉 (NaOCl) 進行表面漂洗 30 s 消毒，再以無菌水漂洗 3 次後，將組織置入無菌水震盪均勻，以移植環沾取懸浮液劃線塗於 NA (Nutrient agar) (Difco Laboratories; Becton, Dickinson and Company, France) 培養基平板上。置於30℃生長箱培養 2–3 d後，挑取單一菌落至新的 NA 培養基平板，重複 3 次以純化細菌。

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圖 1.　田間番茄細菌性葉斑病之病徵。葉片出現墨綠色–深褐色病斑，部分病斑融合成大面積斑塊。

Fig. 1.　Symptoms of bacterial leaf spot of tomato in the field. The leaves showed dark green-dark brown spot and some leaf spots coalesced to blotches.

菸草過敏性反應

將分離自番茄病葉之細菌菌株分別培養於 NA 培養基平板，於 30℃ 培養 1 d 以後，懸浮細菌於無菌水中，以分光光譜儀 (Spectrophotometer, spectronic 70, Bausch & Lomb) 調整懸浮液濃度至吸收值 (A_{600}) 為 0.3 (約 10^8 cfu mL^{-1}) 作為接種源，以注射接種法接種細菌於「萬國士」菸草 (*Nicotiana tabacum* cv. Vam-Hicks.) 葉片內，於室溫觀察葉片有無過敏性反應，將可以引起葉片壞疽反應之菌株以 Tls 編號。

病原性試驗

接種試驗以株高約 25 cm 之普遍栽植的番茄栽培品種「嬌女」及「紅番」為供試植物，供試植物種植於盆口直徑 12 cm 之盆缽，內含栽培介質 (泥炭土、珍珠石及蛭石比例 2 : 1 : 1)。選取南投縣細菌分離株 Tls01、Tls02 及花蓮縣細菌分離株 Tls03 及 Tls04 共 4 分離株為供試菌株，接種方法為先以金剛砂於葉片製造傷口後，以上述的方法製備 10^8 cfu mL^{-1} 濃度之細菌懸浮液，以無動力噴灑法將細菌懸浮液噴灑至供試植物葉片，噴灑無菌水之處理作為對照組。接種後植株套上透明塑膠袋保濕 48 h，之後除去塑膠袋置 28℃ 生長箱觀察，每 1 供試菌株接種 3 棵，並紀錄病徵發展情形，之後再從接種之番茄罹病葉部組織分離細菌，確認是否與原接種之細菌相同。

Biolog Identification System 鑑定細菌

將上述供試菌株培養於 BUGTM Agar (每 1 L 水含 57 g Biolog Universal Growth Agar, Biolog Inc., CA, USA) 培養基約 16–24 h，重複劃線培養於 BUGTM Agar，經 24 h 後，將細菌懸浮於 GN/GP-IF 緩衝液 (0.4% NaCl; 0.03% pluronic F-86; 0.01% gellan gum)，調整細菌懸浮液濃度至 63%T (Turbidity)，將懸浮液加入 Biolog GN2 反應盤 (Biolog Inc., CA, USA) 中，每孔加入 150 μL 懸浮液，置於 30℃ 下培養 16–24 h 後以光譜儀測讀，所得資料經 Biolog GN 資料庫 (Biolog 6.01 版) 比對，以鑑定細菌種類。

細菌形態觀察與生理生化特性測定

將純培養於 NA 培養基 2 d 之供試菌株，加入少量無菌水，之後輕輕吸取少量菌液滴於蠟膜 (Parafilm, Bemis Co., WI, USA) 上，並加入等量 2% 鎢酸染液 (pH 7.0)，之後將覆有 formvar 支持膜之銅網 (200 mesh) 覆蓋於菌液上 3 min 後，以濾紙吸取多餘染液。再將銅網置於真空乾燥器內，以真空抽氣幫浦抽乾水分後，以穿透式電子顯微鏡 (Hitachi-7000) 觀察細菌形態與鞭毛。生理生化特性的測定依 Schaad *et al*. (2001) 所編著之植物病原細菌鑑定實驗指南所述，進行革蘭氏染色 (Gram staining)、氧化/發酵試驗 (O/F test)、King's B 培養基上螢光色素的形成、YDC (Yeast extract-dextrose-$CaCO_3$) 培養基上之菌落型態、結晶紫果膠 (Crystal violet pectate) 培養基上果膠分解能力測定、40℃ 下之生長能力、果聚醣 (Levan) 的生成、氧化酵素 (Oxidase test) 測定、馬鈴薯致腐測試 (Potato soft rot)、精氨酸二水解酶 (Arginine dihydrolase) 測定、明膠 (Gelatin) 液化能力測定，對 Mannitol、Cellobiose、Sorbitol、Sucrose、Trehalose 的利用能力，以鑑定其分類地位。

16S rDNA 定序

選取於 NA 培養之 Tls01 及 Tls03 菌株，

參考 Wang *et al*. (1993) 之簡易方法並略爲修改，以滅菌牙籤沾取經純化後之單一菌落，放入 50 µL 之無菌水中懸浮，再加入 50 µL 之 0.4 N NaOH 靜置 10 min 後，再加入 1 M Tris-HCl 100 µL 混合均勻。取 20 µL 加入無菌水中稀釋 10 倍，再取 2.5 µL 爲模版，再以細菌 16S rDNA universal primers f8-27/r1510 (Lipson & Schmidt 2004) 進行 PCR 增幅。增幅後之產物以 1.4% agarose 進行電泳分析後，將預期的 DNA 片段以 TOPO TA cloning Kit (Invitrogen Corporation, CA, USA) 進行選殖。所得之選殖株經增殖後抽取質體，再以 PCR 確認所選殖片段爲預估長度後，委由明欣生物科技有限公司 (Mission Biotech, Taipei, Taiwan) 進行定序工作。

細菌單一菌落快速鑑定

以上述粹取核酸法抽取供試菌株核酸後，進一步以 *P. cichorii* 專一性引子對 SfL1/SfR2，配合 PCR 需求條件進行 (Hseu *et al*. 2006)，此引子對可對 *P. cichorii* 增幅出 379-bp 之 DNA 片段。PCR 反應在 20 µL 混合液中進行，分別加入 2 µL 之 10 × PCR buffer、0.5 µL 之 10 mM dNTP、0.8 units 之 *Taq* DNA polymerase 及各 0.5 µL之10 µM SfL1 與 SfR2 引子對，於熱循環反應器 (GeneAmp PCR System 2400) 內進行，溫度循環條件爲 94℃進行 5 min、94℃進行 1 min、5℃ 30 s、72℃ 30 s，共 30 cycles。再以 72℃進行 10 min，最後將增幅產物以 1.4% agarose gel 進行電泳分析。

藥劑感受性測定

隨機選取 Tls04 細菌分離株，進行藥劑感受性試驗，以濾紙圓盤擴散法 (paper disc diffusion method) (Adaskaveg & Hine 1985) 於 NA 培養基測試供試菌株對不同藥劑於不同濃度之感受性。各菌株濃度爲 10^8 cfu mL^{-1}，分別取 0.1 mL 菌液混合於 6 mL 之水瓊脂 (Water agar) 中，再覆於 NA 平板上。將已稀釋成不同濃度的藥劑各 0.1 mL 滴入直徑 13 mm 之濾紙圓盤 (Whatman International Ltd.) 中，每皿 NA 培養基上放置 3 片含藥之濾紙圓盤，並以滴入無菌水之濾紙圓盤爲對照組。將含有濾紙圓盤的細菌培養皿，於 30℃ 下培養 48 h 後，測量抑制圈大小，以測定藥效。供試藥劑種類包括 10 種市售藥劑，計有抗生素類之鏈四環黴素 (Streptomycin Tetracycline，商品名爲枯萎寧，10.0% SP，全台公司)、鏈黴素 (Streptomycin，商品名爲立農黴素，12.5% SL，立農公司)、嘉賜黴素 (Kasugamycin，商品名爲菌友，2.0% WP，世大化工)；市售含銅類藥劑之鹼性氯氧化銅 (Copper oxychloride，商品名爲新必利丹，70.0% WP，青山公司)、氫氧化銅 (Copper hydroxide，商品名爲克菌多，53.8% WG，台灣杜邦公司)；三元硫酸銅 (Tribasic copper sulfate，商品名爲果太保，27.12% SC，嘉濱貿易公司)；市售含鋅錳類藥劑之鋅錳乃浦 (Mancozeb，商品名爲興農生-45，80.0% WP，興農公司)；市售混合類藥劑之嘉賜銅 (Kasugamycin + Copper oxychloride，商品名爲加速黴素，81.3% WP，世大化工) 及多保鏈黴素 (Thiophanate methyl + Streptomycin，商品名爲萬億黴素，68.8% WP，瑞芳化工)；市售其他類藥劑之歐索林酸 (Oxolinic acid，商品名爲金星，20.0% WP，台灣住友公司)。供試藥劑有效成分濃度爲 40、200 及 1,000 mg L^{-1}，每處理 3 皿，並以滴入無菌水之濾紙圓盤爲對照組。

結果

過敏性反應與病原性

將萬國士煙草以注射接種細菌懸浮液 24 h 後，葉片即出現壞疽之過敏性反應。於病原性測試之接種試驗中，供試菌株 Tls01–02 (分離自南投縣)，Tls03–04 (分離自花蓮縣)，以噴灑接種細菌懸浮液於供試嬌女及紅番番茄植株，4 供試菌株接種結果相同，於接種 24 h 後均可於葉部造成水浸狀之墨綠至深褐色之小斑點病徵，48 h 以後水浸狀斑點擴大，部分斑點融合成斑塊狀病斑 (圖 2)，與田間所見病徵相似。由所接種植株之病斑處可再分離出相同的細菌。接種無菌水之對照植株則無病徵出現。

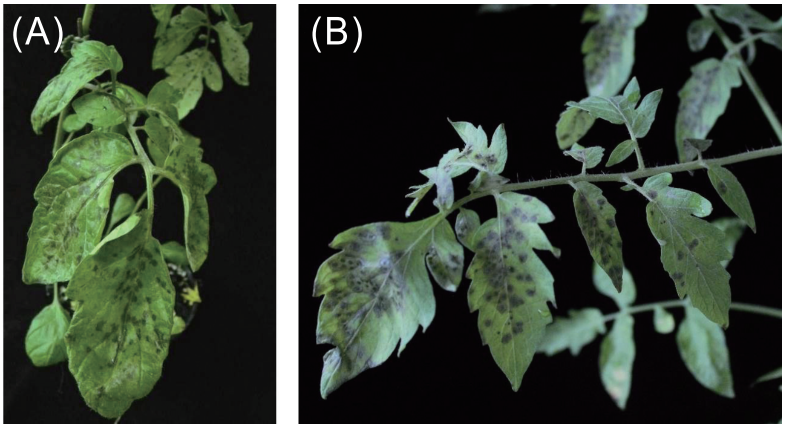

圖 2.　番茄人工機械接種分離到的細菌後呈現的病徵。(A)「嬌女」番茄與 (B)「紅番」番茄接種後均出現墨綠至褐色的水浸狀斑點。

Fig. 2.　Symptom appearance of the tomato inoculated with the isolated bacteria. Two tomato cultivars (A) 'Jiao Nu' and (B) 'Hong Fan' showed dark green-dark brown water-soaking leaf spots after inoculation.

細菌形態與生理生化測定

經於電子顯微鏡下觀察供試菌株形態，為極生多根鞭毛之桿狀細菌。以革蘭氏染色結果，屬於革蘭氏陰性 (Gram negative)。以氧化方式利用葡萄糖在NA培養時，菌落為白色；在 King's B 培養基上，可產生螢光色素；在結晶紫果膠 (Crystal violet pectate; CVP) 培養基，不形成凹陷、不產生果聚醣 (Levan)，具氧化酶 (Oxidase) 而不具精氨酸二水解酶 (Arginine dihydrolase)，無法液化白明膠 (Gelatin)；在馬鈴薯組織塊上，不具致腐能力；又於 40℃ 下不生長。可利用 Mannitol，不能利用 Cellobiose、Sorbitol、Sucrose 及 Trehalose (表 1)。

Biolog system 鑑定結果

將供試菌株 Tls01–04 共 4 株，於 Biolog Identification System 分析 4 菌株對 95 種碳素源的利用情形，將試驗所得資料於 Biolog GN 資料庫 (Biolog 6.01 版) 比對顯示各供試菌株均屬於 *P. cichorii* (*syringae*)，相似值依序為 0.97、0.72、0.89 及 0.93。

16S rDNA 定序及鑑定

選取 Tls01 與 Tls04 供試菌株，以細菌 16S rDNA f8-27/r1510 引子對進行 PCR 增幅，均可增幅出一約 1500-bp 之 DNA 片段。此 DNA 片段經選殖及定序後，菌株 Tls01 (NCBI Accession KJ567157) 與 Tls04 (NCBI Accession KJ567158) 於 NCBI (National Center for Biotechnology Information) 基因資料庫分析比對，比對結果供試菌株均與 *Pseudomonas cichorii* MAFF211996 strain 及 PPST50936 strain (NCBI Accession Number: AB724282.1 及 JQ994483.1) 之 16S rDNA 序列相同度達 99% 以上。

專一性引子對鑑定單一菌落

為了進一步確認此分離菌株為 *P. cichorii*，挑取培養於NA培養基之單一菌落，以對 *P. cichorii* 具專一性之引子 SfL1/SfR2 進行 PCR 反應，於電泳分析後，各供試菌株均可增幅出 *P. cichorii* 特異性之 379-bp DNA 片段，與對照菌株 *P. cichorii* (BCRC 編號 12682) 增幅的片段大小一致 (圖 3)。

藥劑感受性測定

以濾紙圓盤擴散法，於 NA 培養基上，測定番茄葉斑菌株 Tls04 對不同藥劑於不同濃度下之感受性。於 10 種藥劑在 3 種有效成份濃度 40、200、1,000 mg L^{-1} 下，測試對該病菌生長之抑制效果，當藥劑有效成份濃度僅 40

表 1. 番茄細菌性葉斑病菌之生理生化分析。

Table 1. Physiological and biochemical analysis on tomato bacterial leaf spot pathogen.

Character	Strains from tomato	*P. cichorii*[z]	*P. syringae*
Gram reaction	G (−)[y]	G (−)	G (−)
O/F test[y]	O	O	O
Fluorescent pigment on KB	＋	＋	＋
Diffusible non-fluorescent pigment on KB	－	－	－
Levan	－	－	＋
Oxidase	＋	＋	－
Pectolytic Activity	－	－	－
Arginine dihydrolase	－	－	－
Gelatin hydrolysis	－	－	V
Colonies yellow on YDC	－	－	－
Colonies mucoid on YDC at 30℃	－	－	－
Growth at 40℃	－	－	－
Utilization of:			
Mannitol	＋	＋	V
Cellobiose	－	－	－
Sorbitol	－	－	＋
Sucrose	－	－	＋
Trehalose	－	－	－
Tobacco HR	＋	＋	＋

[z] Data are from Schaad *et al*. 2001.
[y] G (−), Gram negative; O, oxidative; F, fermentation; ＋, postive; －, negative; v, 21–79% postive.

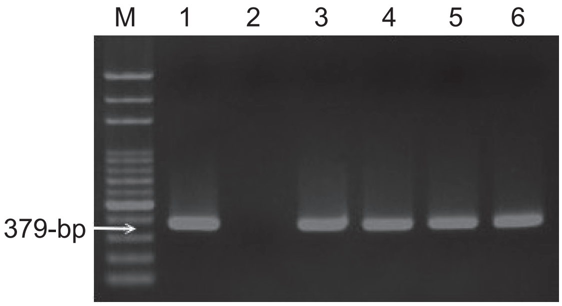

圖 3. 以引子對 SfL1/SfL2 配合 PCR 技術鑑定番茄細菌性葉斑病菌。M 為 100-bp Marker，1 為 *Pseudomonas cichorii* (BCRC 編號 12682)，2 為無菌水之負對照，3–6 為葉斑病菌 Tls01–04。

Fig. 3. Specific amplification of tomato leaf spot pathogen by polymerase chain reaction with specific primer pairs SfL1/SfL2. Lane M, 100-bp DNA marker; lane 1, DNA from *Pseudomonas cichorii* (BCRC number 12682); lane 2, ddH2O for negative control; and lanes 3–6, DNA from tomato leaf spot pathogen.

mg L^{-1}，鏈四環黴素及鏈黴素即產生抑制圈，抑制圈半徑 3.30–5.17 mm；當藥劑有效成份濃度為 200 mg L^{-1}，包含上述 2 種藥劑，嘉賜黴素、多保鏈黴素及歐索林酸亦出現抑制圈，半徑 1.27–9.77 mm；濃度為 1,000 mg L^{-1} 時，出現抑制圈之藥劑種類仍維持為鏈四環黴素、鏈黴素、嘉賜黴素、多保鏈黴素及歐索林酸，半徑 3.23–15.10 mm。其餘藥劑包括鹼性氯氧

化銅、氫氧化銅、三元硫酸銅、嘉賜銅、鋅錳乃浦，則均對供試菌株無抑制效果 (表 2)。

討論

2010–2011 年時於南投縣與花蓮縣等番茄栽培區，發現部分番茄葉片出現墨綠－深褐色之圓形或不規則形斑點，經病原菌分離及接種試驗確認此問題為細菌所引起。此病原菌經測試為革蘭氏陰性，菌體為桿狀具極生數根鞭毛；屬好氣性細菌，於 YDC (yeast extract-dextrose-$CaCO_3$) 平板培養時，菌落為白色，不具黏稠狀；培養在 King's B 平板時會產生螢光色素；於 40℃ 不生長，由這些特性比對 Schaad (2001) 所述資料，顯示此細菌屬於 *Pseudomonas* 屬。以 Biolog 鑑定系統測試供試 4 株番茄葉斑細菌結果為 *Pseudomonas cichorii* (*syringae*) 相似度在 07–1.0 之間，顯示此病菌可能為 *Pseudomonas cichorii* 或 *Pseudomonas syringae*。依 Schaad *et al*. (2001) 之細菌鑑定資料，上述 2 種細菌可由其他生理生化特性加以區分，因此進一步測試供試菌株之生理生化特性發現該病菌具有氧化酶作用、不產生果聚醣、不利用 Sorbitol 及 Sucrose 之特性與 *P. cichorii* 相同但與 *P. syringae* 不同 (表 1)，因此該菌應與 *P. cichorii* 較為接近。又於分子鑑定上，16S rDNA 序列與 *P. cichorii* 相似度達 99% 以上，應用 *P. cichorii* 專一性引子 SfL1/SfR2 測試供試細菌時，可增幅出特異性之 379-bp 片段，因此依據上述資料鑑定該菌為 *P. cichorii*。

P. cichorii 又稱為菊苣假單胞菌，最早在菊苣 (Chicory) 上發現 (Swingle 1925)，該病菌寄主範圍廣泛，可危害多種蔬菜作物及觀賞植物，如芹菜 (Celery) (Wilk & Dye 1974)、番茄 (Tomato) (Wilk & Dye 1974)、萵苣 (Lettuce) (Grogan *et al*. 1977)、甘藍 (Cabbage) (Smith & Ramsey 1956)、菊花 (Chrysanthemum) (Jones *et al*. 1983)、天竺葵 (Geranium) (Engelhard *et al*. 1983)、木蘭花 (Magnolia) (Mullen & Cobb 1984)、鵝掌藤 (Dwarf scheflera) (Chase & Brunk 1984) 等。此菌於台灣曾在 1988 年被發現引起芹菜葉斑病 (Su 1988)，之後此病菌陸續被報導可造成向日葵細菌性葉斑病 (Hseu *et al*. 2005) 及萵苣細菌性葉腐病 (Hseu *et al*. 2009) 等病害，但未見於番茄作物。因此，本篇報告為番茄可被 *P. cichorii* 感染在台灣之首次紀錄，並依據其所造成之葉斑病徵將此病稱之為番茄細菌性葉斑病 (bacterial leaf spot)。

表 2. 供試藥劑在不同濃度對番茄葉斑菌株 Tls04 之生長抑制效果。

Table 2. Growth inhibition of *Pseudomonas cichorii* Tls04 by various agrochemicals in different concentrations.

	Inhibition zone (mm in diam.)		
Chemical	40 mg L^{-1}	200 mg L^{-1}	1,000 mg L^{-1}
Streptomycin + Tetracycline (10% SP)	5.17 ± 0.03 a[z]	9.77 ± 0.15 a	15.10 ± 0.10 a
Streptomycin (12.5% SL)	3.30 ± 0.25 b	6.67 ± 0.18 b	9.57 ± 0.23 b
Kasugamycin (2.0% WP)	0 c	4.20 ± 0.12 c	9.23 ± 0.14 c
Thiophanate methyl + Streptomycin (68.8% WP)	0 c	2.43 ± 0.19 d	5.43 ± 0.07 d
Oxolinic (20% WP)	0 c	1.27 ± 0.03 e	3.23 ± 0.09 e
Kasugamycin + Copper oxychloride (81.3% WP)	0 c	0 f	0 f
Copper hydroxide (77.0% WP)	0 c	0 f	0 f
Tribasic copper sulfate (27.12% SC)	0 c	0 f	0 f
Copper oxychloride (85.0% WP)	0 c	0 f	0 f
Mancozeb (80.0% WP)	0 c	0 f	0 f
$LSD_{0.05}$	0.24	0.30	0.29

[z] Mean ± standard error (n = 3). Means within each column followed by the same letter(s) are not significantly different at 5% level by LSD test.

依本次接種試驗顯示，此病菌可經由菌液噴灑植株葉片，經由金剛砂製造的微小傷口感染番茄植株，故可推測此病於田間可能經由風沙造成葉片傷口後，病菌經雨水或噴灑澆灌飛濺至健康葉片從傷口入侵。因此，防治上應注意風大區域的防風措施，以減少風沙造成葉片傷口，及避免以灌溉噴灑方式以減少感染。在藥劑防治上，以花蓮縣分離株 Tls04 於室內篩選可抑制病菌生長的藥劑，發現全部 10 種供試藥劑中以抗生素類藥劑之鏈四環黴素抑制細菌生長之抑制圈顯著最大 (P < 0.05)、鏈黴素次之、嘉賜黴素再次之，然而於 3 種含銅類藥劑包括氫氧化銅、三元硫酸銅及鹼性氯氧化銅等藥劑於所測試的 3 種濃度均無法抑制此菌的生長，顯示田間已存在對銅劑具高忍受度菌株。此結果與南投縣分離株之測試結果無明顯差異 (資料未列出)，因此應避免含銅類藥劑在田間頻繁施用，以防止抗藥性問題。

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Occurrence of Bacterial Leaf Spot of Tomato Caused by *Pseudomonas cichorii* in Taiwan

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Abstract

Tsai, C. H., P. J. Ann, Y. S. Lu, M. D. Chen, S. L. Hwang, and Y. H. Peng. 2014. Occurrence of bacterial leaf spot of tomato caused by *Pseudomonas cichorii* in Taiwan. J. Taiwan Agric. Res. 63(2):143–150.

During 2010–2011 an unknown disease of tomato was found in Nantou and Hualien counties. Dark green to dark brown irregular or round spots were observed on the diseased leaves. The bacteria isolated from the infected tissues can induce tobacco hypersensitive reaction and their pathogenicity was verified by Koch's postulates. The pathogen was further identified as *Pseudomonas cichorii* based on physiological and biochemical tests, Biolog identification, 16S rDNA sequence analysis, and specific primers Sf1/SfR2 following polymerase chain reaction. This is the first report of *P. cichorii*-elicited tomato bacterial leaf spot disease in Taiwan. Among the tested agrochemicals, the mixture of streptomycin and tetracycline has the highest efficacy in inhibiting bacterial growth.

Key words: Tomato, Bacterial leaf spot, *Pseudomonas cichorii*, Agrochemical screening.

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使用 Real-time PCR 檢測引起西瓜蔓枯病菌的方法評估

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摘要

關政平、黃彥華、曾清山、林宗俊、石信德。2014。使用 Real-time PCR 檢測引起西瓜蔓枯病菌的方法評估。台灣農業研究 63(2):151–158。

本研究根據西瓜蔓枯病菌 (*Didymella bryoniae*) 的 rDNA 設計出專一性的引子對及利用 real-time PCR 法與 TaqMan 螢光探針進行 *D. bryoniae* 之檢測並與傳統 PCR 法作比較，結果顯示 real-time PCR 法較傳統 PCR 法靈敏度提高約 100 倍。利用 real-time PCR 法偵測被 *D. bryoniae* 在感染的西瓜組織，結果發現 *D. bryoniae* 主要分布在西瓜的莖部。本研究使用 real-time PCR 法可增幅出 *D. bryoniae* 的專一性核酸片段，並檢測感染西瓜組織及種子中之 *D. bryoniae* 核酸，將可提供西瓜蔓枯病之病害檢測與病菌量化之判定。

關鍵詞：西瓜蔓枯病、*Didymella bryoniae*、Real-time PCR、診斷技術。

前言

蔓枯病 (gummy stem blight) 係由子囊菌 *Didymella bryoniae* (Fuckel) Rehm [*Mycosphaerella citrullina* (C. O. Sm.)] 所引起的葫蘆科作物之病害。*D. bryoniae* (無性世代為 *Ascochyta cucumeris* Fautr. & Roum.) 會感染包括有西瓜、甜瓜、南瓜及胡瓜等葫蘆科作物的莖、葉、花及果實，罹病之瓜類葉片、葉柄及莖部會有褐色病變，所產生子囊孢子及柄孢子再經由風及雨水傳播至其他植株 (Sitterly & Keinath 1996)。果實及花卉受到感染後，會導致果實內部腐爛並且使其種子遭到污染 (Keinath *et al*. 1995)。此外，此病菌可存活於種子、空氣及土壤中 (Keinath 2011)，並在溫暖和潮濕的天氣條件下，能迅速殺死葫蘆科作物的藤蔓，產生蔓枯的現象 (Bala & Hosein 1986; McGrath *et al*. 1993; Sitterly & Keinath 1996)。

目前廣泛用於檢測種子是否帶有 *D. bryoniae* 之方法為種苗出芽 (grow-out) 或種子轉漬 (blotter assays) 試驗。這些方法須具有廣大的育苗場地，直接利用肉眼觀察種苗之病徵與發病情形或利用鏡檢等技術加以判斷，惟此等方法需要時間、空間、密集的勞力及需有對病原菌之專業知識才能判斷病徵 (www.seedhealth.org)。綜觀過去在種苗的檢測方法，已有諸多利用 PCR 方法進行檢測的研究報告：如以 PCR 檢測甘藍種子是否帶有 *Alternaria radicina* (Pryor & Gilbertson 2001) 及西瓜果斑病病害 (Ha *et al*. 2009)；使用 real-time PCR 檢測洋蔥種子是否帶菌 *Botrytis* spp. (Chilvers *et al*. 2007) 等。西瓜是世界上重要的瓜類作物之一，在 2008 年全世界種植西瓜的面積約有 3,752,568 ha，產量約有 99,194,223 Mg (FAO 2008)。而在台灣，其種植面積約有 12,665 ha，居所有蔬菜作物之冠 (COA 2008)。許多由真菌類所引起的疾病，會使西瓜產量大幅減少，其中由 *D. bryoniae* 所引起的蔓枯病則為西瓜最嚴重的病害之一。

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因此，本研究的目的在於開發專一性強、靈敏度高及快速的 real-time PCR 方式，藉以確認西瓜種苗是否帶有 *D. bryoniae*，為防治此病害提供一個有效的鑑定工具。

材料與方法

植物總量 DNA 之純化

將西瓜組織切下，用液態氮急速冷凍後研磨成粉，取 0.1 g 研磨樣品用 Wizard® Genomic DNA Purification Kit (Pormega, USA) 純化出總量 DNA，保存於 -80℃ 備用。

病原菌來源及菌絲總量 DNA 之純化

本研究使用之菌種與參考菌種，包括獲自食品工業研究所、嘉義大學蔡竹固教授、中興大學曾國欽、黃振文教授等及自行分離之菌株，如表 1。所有供試菌株皆經單孢分離並培養於馬鈴薯葡萄糖瓊脂培養基 (PDA, Potato dextrose agar, Difco, USA)。供試菌株培養至 PDA 培養基並於 25℃ 下培養 8–14 d 後。以去離子水將供試菌株之菌絲刮洗下來，並以 13,000 rpm 離心 2 min，除去上清液後，收菌絲用液態氮急速冷凍研磨成粉，備用。取 0.1 g 磨碎之菌絲樣品以前述之試劑套組純化出供試菌株之總量 DNA，並保存於 -80℃ 備用。

引子設計

利用 Primerplex 2.0 (USA) 軟體，針對 *D. bryoniae* 的 rDNA ITS 核酸序列及植物核酸序列進行引子設計。根據所獲得的序列，找出數個保留性高的區域，設計出對應之專一性引子 (表 2)，供檢測使用。即時螢光定量所使用的螢光探針是來自 Roche Applied Science 的 probe#89 (Roche, USA)。此螢光探針是一段專一性結合於 rRNA 的基因序列，探針的 5’ 端標定 FAM (6-carboxyfluorescein acronym fam)，其 3’ 端標定 BHQ-1 (Black Hole Quencher-1)，每合成一次新鏈就有一次螢光信號釋放，所發出的綠色螢光利用儀器偵測每次聚合酶鏈鎖反應循環後產物總量。

聚合酶鏈鎖反應

PCR 所使用的藥劑為 Super-Run Taq DNA Polymerase (Protech, PT-53-250U)。於 0.2 mL 微量離心管中分別加入去離子水 18.5 μL、10× PCR buffer 2.5 μL、10 mM dNTP 0.5 μL、

表 1. 使用 real-time PCR 及 PCR 檢測 *Didymella bryoniae* 分離株及西瓜常見病原菌之結果。
Table 1. Specificity of real-time PCR and conventional PCR assay for the detection of watermelon pathogens.

Fungal/bacterium	Code	Host	Source[z]	PCR[y]	Q-PCR[x]
Acidovorax avenae subsp. *citrulli*	AAC33	Watermelon	Kuo-Chan Tzeng	−	−
Colletotrichum orbiculare	E0702	Watermelon	Jwu-Guh Tsay	−	−
Glomerella magna	A0601	Watermelon	Jwu-Guh Tsay	−	−
Rhizoctonia solani	AGI-IB	Rice	Jenn-Wen Huang	−	−
Colletotrichum higginsianum	PA01	Cabbage	Jenn-Wen Huang	−	−
Colletotrichum nigrum	BCRC 35149	Watermelon	BCRC	−	−
Colletotrichum lagenarium	BCRC 35030	Watermelon	BCRC	−	−
Fusarium spp.	GL0905-2 (F8)	Watermelon	Jwu-Guh Tsay	−	−
Didymella bryoniae	DB-10	Watermelon	Jwu-Guh Tsay	+	+
Ascochyta cucumeris	BCRC 35163	Watermelon	BCRC	+	+
Ascochyta cucumeris	BCRC 35243	Watermelon	BCRC	+	+
Didymella bryoniae	D1-D10	Watermelon	This study	+	+

[z] BCRC is the Bioresource Collection and Research Center, Hsin-Chu, Taiwan; Dr. Jwu-Guh Tsay, Department of Nutritional Science, Chiayi University, Chiayi, Taiwan; Drs. Kuo-Chen Tzeng and Jenn-Wen Huang, Department of Plant Pathology, National Chung Hsing University, Taichung, Taiwan.
[y] Detection of the PCR product on 3% agarose gel; +, PCR product detected; −, no product detected.
[x] (+) indicates a cycle threshold (Ct) < 35; (−) indicates a Ct > 35.

表 2. 本試驗所使用之引子與核酸探針。

Table 2. Primers and probes used in this study.

Oligonucleotide	Sequence (5'-3')	Temp. (℃)	Amplicon (bp)	Modification
Di-real-61-F1	ACCAGCCTGGACTGAGGTC	60	61	
Di-real-61-R1	GGCCGCTTACAGCCATTA	60	61	
89 probe[z]	GGATGCTG			5'-FAM 3'-BHQ1

[z] FAM: 6-carboxyfluorescein acronym fam, BHQ-1: Black Hole Quencher-1.

D. bryoniae 正向引子 (Di-real-61-F1) 1 µL、*D. bryoniae* 反向引子 (Di-real-61-R1) 1 µL、Taq Polymerase 及 5 µM 待測西瓜或其病原菌的總量 DNA 1 µL，總體積 25 µL 進行 PCR 反應。反應條件為 94℃ 持續 3 min；95℃ 持續 30 s、60℃ 持續 30 s、72℃ 持續 30 s，共 35 個循環 (cycle)；最後再以 72℃ 反應 7 min。PCR 反應的機器為 Veriti® Thermal Cycler (Applied Biosystems, USA)，最後取 5 µL PCR 產物進行 3% 膠體電泳，經由 EtBr 染色後，以 UV 光照相並分析結果。

即時螢光定量聚合酶鏈鎖反應

Real-time PCR 所使用的試劑為 FastStart Universal Probe Master (Rox) (Roche Applied Science)。於 0.2 mL 微量離心管中分別加入去離子水 6.8 µL、5 µM 待測西瓜或其病原菌的總量 DNA 1 µL、2× FastStart Universal Probe Master (Rox) 10 µL、10 µM *D. bryoniae* 正向引子 (Di-real-61-F1) 1 µL、10 µM *D. bryoniae* 反向引子 (Di-real-61-R1) 1 µL 及 Roche#89 probe 0.2 µL，總體積 20 µL 進行 PCR 反應。反應條件如下：50℃ 持續 5 min、95℃ 持續 3 min 和 40 個循環的 95℃ 持續 3 s、60℃ 持續 1 min。real-time PCR 反應的機器為 ABI PRISM® 7500 Sequence Detection System (Applied Biosystems, USA)。

偵測靈敏度試驗

為測定所設計的探針序列之靈敏度，將萃取自 *D. bryoniae* 菌絲或分生孢子的總量 DNA 以 10 倍序列稀釋後，利用傳統 PCR 及 real-time PCR 進行測試。將 *D. bryoniae* 總量 DNA 從 50 ng 至 50 fg 的負對數值 (-log) 與即時螢光聚合酶反應後得到相對應的 Ct 值，製作標準曲線方程式；即縱座標為 Ct 值，橫坐標為總量 DNA 濃度的負對數值 (-log)，每個數據點代表由 3 次獨立試驗所得到得平均 Ct 值。線代表 DNA 濃度與 Ct 值之間的線性迴歸分析。

西瓜組織中 *D. bryoniae* 之定量分析

為測定 *D. bryoniae* 在西瓜組織中分佈的情形，將西瓜幼苗 (株高約為 25–30 cm) 之 2 片下位葉，噴灑 *D. bryoniae* 之分生孢子懸浮液 (1×10^6 spores mL^{-1})，將西瓜苗套袋並放置於陰暗處 1 d 後，打開套袋並移入溫室中持續培養約 2 wk，待西瓜幼苗葉片出現病斑後。取罹病之西瓜的不同部位 (根、莖、上位葉、側芽、下位葉) 及植株周邊土壤進行總量 DNA 萃取，並調整樣品之總量 DNA 濃度為 50 ng，每個反應 3 重複，以 real-time PCR 進行定量分析。此外，本研究將 *D. bryoniae* 之人工帶菌西瓜種子分作 3 個試驗組 (A1、A2、A3) 及未帶菌之健康西瓜種子 (B1、B2、B3) 進行檢測，每組各取兩顆種子進行純化總量 DNA，同時以傳統 PCR 與 real-time PCR 分析。

結果

D. bryoniae 專一性試驗

為確定所設計的引子對及探針之專一性，首先以 PCR 方法針對西瓜常見病害之病原菌 (表 1) 進行檢測，再由瓊脂凝膠電泳分析，經瓊脂凝膠電泳分析的結果顯示僅在 DB10、BCRC35163 及 BCRC 35243 等 *D. bryoniae* 菌株出現大小 61 bp 產物之核酸片段，對其他類

病原菌包括*Acidovorax avenae* subsp. *citrulli*、*Colletotrichum lagenarium*、*Colletotrichumorbiculare*、*Colletotrichumhigginsianum*、*Colletotrichumnigrum*、*Fusarium* spp.、*Glomerella magna* 及 *Rhizoctonia solani* 等， 以及作爲負對照組之健康的西瓜與無菌水皆無 PCR 產物生成，可初步確定此引子對 *D. bryoniae* 具有專一性。相同的樣品使用 real-time PCR 分析，結果顯示僅在 D1–D10、DB10、BCRC35163 及 BCRC 35243 等 *D. bryoniae* 菌株皆爲正反應 (表 1)，對於前述其他類病原菌以及健康的西瓜與作爲負對照組之無菌水皆無反應 (表 1)。由上述兩個試驗結果顯示，本研究設計的引子對和探針在傳統 PCR 及 real-time PCR 之測試中均對 *D. bryoniae* 具有高度專一性。

D. bryoniae 靈敏度試驗

爲了得知試驗方法對 *D. bryoniae* 的靈敏度，使用純化自 *D. bryoniae* 菌絲的總量 DNA (50 ng) 作爲試驗用之核酸模板，經 10 倍系列稀釋後，利用傳統 PCR 法及 real-time PCR 法進行分析，並比較兩種檢測法靈敏度的差異。根據 real-time PCR 結果並設定 Ct 值爲 35 以內爲陽性反應，結果顯示 *D. bryoniae* 菌絲的總量 DNA 可被偵測的最低量爲 0.5 pg (圖 1A)。若將傳統 PCR 法的產物經瓊脂凝膠電泳分析，可增幅出 61 bp 之 *D. bryoniae* 之專一性核酸片段 (圖 1C)，結果顯示 *D. bryoniae* 菌絲的總量 DNA 可被偵測的最低量爲 50 pg。由上述結果，顯示利用real-time PCR法檢測*D. bryoniae* 菌絲的總量 DNA 之靈敏度高於傳統 PCR 法達 100 倍。將上述 real-time PCR 所測試之 *D. bryoniae* 菌絲總量 DNA 經由 10 倍系列稀釋進行靈敏度試驗，並將其結果繪製標準曲線，縱座標爲 Ct 值，橫坐標爲 *D. bryoniae* 菌絲總量 DNA (*D. bryoniae* 菌絲的總量 DNA 經 10 倍系列稀釋，從 50 ng 至 5 fg) 的負對數值 (-log)，*D. bryoniae* 菌絲總量 DNA 的標準曲線方程式爲 $Y = 3.034X + 17.663$；$R^2 = 0.999$ (圖 1B)。根據繪製之標準曲線的結果顯示準確性及 Ct 與螢光值相關性佳。

西瓜不同組織帶菌量試驗

爲了得知當西瓜被 *D. bryoniae* 感染時，此病菌在植株上的分佈情形，將罹病之西瓜植株的側芽、上位葉、下位葉、葉脈、莖、根及根部附近的土壤純化其總量 DNA 後，以 real-time PCR 法分析，並將所偵測到的 Ct 值帶入由靈敏度試驗繪製而成的標準曲線公式中，可推得各部位的 *D. bryoniae* 總量 DNA 的含量，分別爲側芽組織 0.42 pg、上位葉 0.07 pg、下位葉 0.31 pg、葉脈 0.09 pg 及莖 1,208 pg (圖 2)，此外，根、根部附近的土壤、及作爲負反應對照組之健康的西瓜與無菌水皆無偵測到螢光訊號。由上述結果，顯示 *D. bryoniae* 主要分布在罹病西瓜植株的莖部，其帶菌量遠較其他受感染部位高，在下位葉及側芽則有少量菌被檢測出來。另外，測試 *D. bryoniae* 之人工帶菌西瓜種子，結果發現利用傳統 PCR 與 real-time PCR 法皆可檢測出 A-1、A-2 及 A-3 三批西瓜種子爲陽性反應，另三批 (B-1、B-2、B-3) 西瓜種子則爲陰性反應 (表 3)，顯示 real-time PCR 與傳統 PCR 法皆可用於檢測受 *D. bryoniae* 污染之西瓜種子。

表 3. 利用傳統 PCR 與 real-Time PCR 檢測 *Didymella bryoniae* 之人工帶菌種子。

Table 3. Evaluation of conventional PCR and real-time PCR for the detection of *Didymella bryoniae* in artificially infested seeds.

		Detection	
Seed sample[z]	Bioassay[y]	PCR	real-time PCR
A-1	+[x]	+	+ (34.43)[w]
A-2	+	+	+ (34.67)
A-3	+	+	+ (34.97)
B-1	−	−	−
B-2	−	−	−
B-3	−	−	−

[z] Seeds provided by Tsung-Chun Lin.
[y] Examined by tissue blotter or light microscopy.
[x] +: Positive result; −: Negative result.
[w] Cycle threshold value.

討論

蔓枯病是一種嚴重的西瓜病害，主要在葉

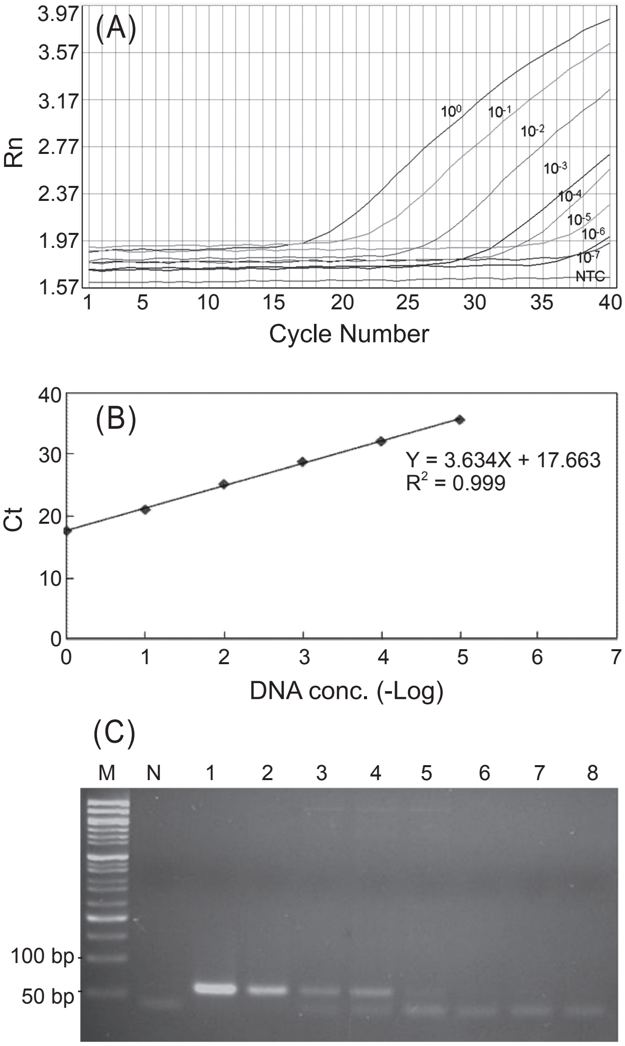

圖 1. 比較 real-time PCR 法 (A, B) 與 PCR 法 (C) 檢測 *Didymella bryoniae* 菌絲總量 DNA 靈敏度試驗。(A) 每條曲線代表稀釋後的總量 DNA 濃度值，縱座標：螢光訊號 (Rn)、橫座標：Cycle Number。(B) *D. bryoniae* 的標準曲線。*D. bryoniae* 總量 DNA 通過即時螢光聚合酶反應後得到相對應的 Ct 值。縱座標：Ct 值，橫坐標：總量 DNA 濃度值。(C) 傳統 PCR 檢測 D. bryoniae 菌絲總量 DNA 靈敏度試驗。Lane1–8 代表稀釋後的總量 DNA 濃度值 (以 10 倍序列稀釋 *D. bryoniae* 總量 DNA 從 5 ng 至 0.5 fg)，N 代表未含有 DNA 模板的負對照。

Fig. 1. Sensitivity comparisons of real-time PCR and conventional PCR assays for the detection of *Didymella bryoniae*. (A) Amplification plot of 10-fold serial dilutions of the total DNA by the real-time PCR. (B) The standard curve of 10-fold serial dilutions of *D. bryoniae* total genomic DNA. (C) Amplification of 10-fold serial dilutions of the total genomic DNA. Lanes1–8, total DNA of *D. bryoniae* infected melon; N, no template control.

和莖上產生病變。雖然果實不一定受 *D. bryoniae* 影響，但當葉片及莖受到感染時，會造成其果實的產量和質量的損失。本研究結果顯示，在西瓜之莖及葉組織均可檢測出 *D. bryoniae* 之存在。目前防治本病的主要方法，包括抗病品種的選育、耕作防治及化學防治，耕作防治包括：確實移除疑似罹病之作物殘株、不保存及不使用疑似感染蔓枯病之種子、瓜類作物與青蔥進行輪作及避免瓜類作物的生產環境保持在潮濕的狀態等；化學防治包括：種子

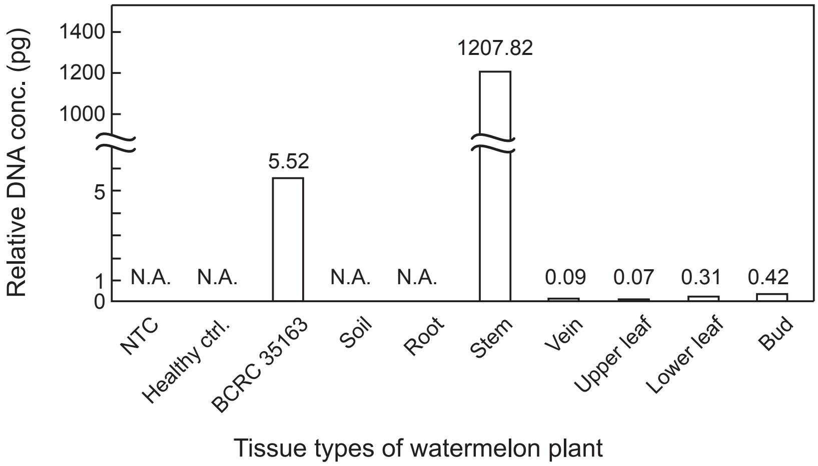

圖 2. 利用 real-time PCR 偵測西瓜不同部位之 *Didymella bryoniae*。檢測樣本包括各部位西瓜組織 (根、莖、上位葉、側芽、下位葉)、土壤、健康西瓜葉片與未含有模板 DNA 之反應。

Fig. 2. Relative quantification of *Didymella bryoniae* by real-time PCR from various parts (root, stem, upper leaf, bud, lower leaf) of watermelon seedlings and those from soil. Healthy ctrl, healthy plant as negative control; NTC, no template control.

催芽前需先泡過消毒液、建議使用 mancozeb 及 benomyl 等防治真菌的藥劑，並避免植栽暴露在有 *D. bryoniae* 的環境下等 (Keinath 1995)。

在蔓枯病菌的檢測上，Somai *et al*. (2002) 使用 PCR 方法檢測 *D. bryoniae* RGI DNA，其靈敏度達 100 pg，另外一個分離菌 *D. bryoniae* RGII 靈敏度達 10 pg。此外，Somai *et al*. (2002) 另發展 PCR 結合免疫分析的 PCR-ELISA 方法檢測 *D. bryoniae* RGI，其雖具有菌體 DNA 定量之優點，但靈敏度僅 10 ng，若以此 PCR-ELISA 方法檢測 *D. bryoniae* RGII 可測到靈敏度則爲 1 ng。Ha *et al*. (2009) 使用 real-time PCR 法檢測 *D. bryoniae*，可測得的 DNA 濃度爲 1 pg，本研究利用 real-time PCR 法可檢測 *D. bryoniae* 菌絲的總量 DNA 的最低量爲 0.5 pg，結果與 Ha *et al*. (2009) 的研究結果相近。本研究、Somai *et al*. (2002)、Ha *et al*. (2009) 檢測的靈敏度皆不盡相同，造成結果不同之原因，除了方法、操作步驟尚包括使用的分離株或不同來源菌株不同等因素。在進行 PCR 試驗部分，本研究利用傳統 PCR 法可偵測到 *D. bryoniae* 菌絲的總量 DNA 的最低量爲 50 pg，雖與 Somai *et al*. (2002) 及 Ha *et al*. (2009) 等人在 PCR 靈敏度的檢測結果略爲不同，但本研究進一步在 real-time PCR 方法的靈敏度試驗較傳統 PCR 方法明顯提升 100 倍以上。許多植物組織特別是種子可能帶有抑制 PCR 之物質而造成偽陰性反應之檢測結果 (DeBoer *et al*. 1995)，如前人在檢測甘藍種子是否帶有 *Alternaria radicina* (Pryor *et al*. 2001) 及洋蔥種子是否帶有 *Botrytis* spp. 之報告 (Chilvers *et al*. 2007)。由本研究結果顯示，由種子萃取核酸並未對所使用之 real-time PCR 或 PCR 方法造成干擾反應之問題，兩者均可檢測西瓜種子是否帶有 *D. bryoniae* 之總量 DNA。

real-time PCR 法所使用的引子對及螢光探針，可專一地偵測到 *D. bryoniae*，且較傳統 PCR 檢測技術具有有更高的靈敏度；另外本法亦具有定量菌量之優點，過去的研究文獻提及受此病原菌感染之果實及花會導致其果實內部腐爛並且感染至種子 (Keinath *et al*. 1995)，且此病害可存活於種子中 (Keinath 2011)，顯示此病菌能經由種子帶菌之可能性很高。本研究所研發之 real-time PCR 方法將可使用於種子檢疫及作物早期診斷之工具，進而採取防治本病害的因應措施。

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Evaluation of Real-time PCR Assay for Detection of *Didymella bryoniae*

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Abstract

Kuan, C. P., Y. H. Huang, C. S. Tseng, T. C. Lin, and H. D. Shih. 2014. Evaluation of Real-time PCR assay for detection of *Didymella bryoniae*. J. Taiwan Agric. Res. 63(2):151–158.

In this study, a specific and highly sensitive real-time PCR assay with TaqMan probe was developed as a tool for the diagnosis of gummy stem blight of watermelon by targeting the rDNA genes of *Didymella bryoniae*. In addition, the real-time PCR assay was compared to a conventional PCR method with their specificity and sensitivity on the detection of *D. bryoniae* in culture andin *planta*. The real-time PCR assay was effective for quantifying the fungal genomic DNA in the infected plants. This method was highly specific to *Didymella* and it was about one hundred times more sensitive than the conventional PCR method. The real-time PCR assaydeveloped isavaluable tool for detection and titer quantitation of gummy stem blight diseases on watermelon.

Key words: Gummy stem blight, *Didymella bryoniae*, Real-time PCR, Detection.

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超高壓液相層析儀快速分析台灣蔬菜葉黃素之新方法

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摘要

羅淑卿、鍾佩恩。2014。超高壓液相層析儀快速分析台灣蔬菜葉黃素之新方法。台灣農業研究 63(2):159–166。

葉黃素可延緩眼睛老化及黃斑部病變，但人體無法自行合成，需由飲食中補充。為建立快速分析葉黃素之方法與台灣常見市售蔬菜葉黃素含量之資料庫，本研究利用超高壓液相層析儀，使用管柱 ethylene bridged hybrid (BEH) C18，以氰甲烷和甲醇 (70 : 30) 之混合液為移動相，等位沖提流洗下，可在 5 min 之內定量標的物葉黃素。此外，α- 及 β-胡蘿蔔素也可利用此方法一併分析定量。本研究分析結果顯示，50 件蔬菜樣本中葉黃素含量介於 0.16–1.77 mg g^{-1} 乾重，其中葉黃素含量大過 1 mg g^{-1} 乾重者有 8 件樣本，多屬深綠色之綠葉蔬菜，包括香菜、空心菜、油菜及芥藍等，其中以香菜葉含量最高，達 1.77 mg g^{-1} 乾重。綠葉蔬菜之葉部含量高於莖部，且皆高於黃色蔬菜及淡色蔬菜，惟淡色蔬菜不被食用之深綠色外葉葉黃素含量大過食用部位。瓜果類蔬菜如大黃瓜，其綠色外皮含量亦高過於白色果肉組織，相差達 2.6 倍。藉由蔬菜外觀色澤和葉黃素含量差異，消費者可選用深綠色之綠葉蔬菜，以增加日常葉黃素攝取量。許多未食用之農業廢棄蔬菜，葉黃素含量高於食用部位，應可進一步作加值利用。

關鍵詞：葉黃素、蔬菜、超高壓液相層析儀。

前言

隨著科技進步，現代人接觸電視、電腦及手機的機會增多，螢幕所放出之藍光會穿透水晶體，造成視網膜光氧化傷害而發炎，致使老年性黃斑部病變提早發生 (aged-related macular degeneration; AMD)，視力會損壞、退化甚至失明。人體血漿中葉黃素 (lutein) 若維持一定濃度，有助於降低 AMD 之發生率 (Schleicher *et al*. 2013)。因爲葉黃素可過濾藍光，避免視網膜受到光引起的氧化傷害 (Junghans *et al*. 2001)，而視網膜黃斑區域之黃斑色素 (macular pigment)，其主要爲葉黃素累積而呈現黃色。此外，葉黃素爲一良好的抗氧化劑，可清除活性氧及自由基，避免其對正常細胞之傷害，並抑制脂質過氧化，因此亦可延緩 AMD 之發生。另外，增加血漿中葉黃素含量，亦可降低化療後頭頸部鱗狀細胞癌 (squamous cell carcinoma) 的危害 (Sakhi *et al*. 2010)。

葉黃素、α-胡蘿蔔素 (α-carotene) 和 β-胡蘿蔔素 (β-carotene) 屬類胡蘿蔔素 (carotenoids)，三者結構相近，皆爲植物色素。人體無法合成葉黃素，必須靠飲食攝入補充 (Semba & Dagnelie 2003)。目前飲食來源有二種，可爲食物或坊間販賣之營養補充品 (Krinsky *et al*. 2003)。隨著生物科技發達，亦有學者著手培育高胡蘿蔔素之小麥品種，希望提高人類攝取機率 (Lachman *et al*. 2013)。

若能建立國產蔬菜中的葉黃素含量與分佈狀況資料庫，作爲一項重要的健康飲食指標，將可提供消費者選擇飲食時之參考依據。本研究擬針對國人常食用之淡色及綠色品種蔬菜進

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行葉黃素含量分析，以建立市售蔬菜葉黃素之含量資料庫。

材料與方法

本試驗由市場收集白色、黃色及深綠色蔬菜作物爲材料，分成莖或葉等不同部位，或是部分需削皮的蔬菜如大頭菜及大黃瓜，則依飲食習慣分成果肉及外皮等可食與未食部位。未食用之外葉乃菜市場菜販所丟棄之深綠色粗大未食用葉，分別進行乾燥磨粉。參考 Khachik *et al*. (1997) 的方法進行葉黃素萃取，萃取前先加入已知濃度的內部標準品 β-apo-8’-carotenal (以方便計算類胡蘿蔔之實際含量)。每一樣本稱取 0.1 g 蔬菜粉，加入 3.7 mL 丙酮及 0.3 mL 去離子水，以超音波震盪機震盪 30 min。以 5,000 ×*g* 離心後，取上清液 2 mL，於 36℃水浴環境下，利用氮氣將上清液吹乾。加入 0.1 mL 乙醚、2 mL 甲醇及 1 mL 15% KOH，振盪混勻後，以同法利用氮氣吹乾最上層的乙醚、甲醇。於 30℃水浴下反應 2 h，期間取出搖晃混勻。加入水 1.5 mL 和正己烷 1.5 mL，振盪混勻 1 min，再加入 2 mL 正己烷，振盪混勻 1 min。將上層移至新的離心管，再加入 2 mL 正己烷，振盪混勻 1 min，將上層移至新離心管。收集 2 次正己烷萃取的有機層，以等體積的水清洗 4 次，吸取上層正己烷以氮氣吹乾，最後加 0.5 mL 氰甲烷回溶。進行定量分析前以 0.2 μm 過濾膜過濾。添加定量之葉黃素、α-胡蘿蔔素及 β-胡蘿蔔素之標準品於分析蔬菜樣本中，以計算其回收率。

葉黃素之定量分析利用超高壓液相層析儀，機型爲 ACQUITY UPLC，分析管柱爲 C18 column (reversed phase chromatographic column,150 mm × 2.1 mm, Waters Co., USA)、檢出器爲光電二極體陣列檢測器 (photodiode array detector; PDA)，檢測波長 450 nm，管柱溫度 40℃。以氰甲烷和甲醇 (70 : 30) 之混合液爲移動相溶液，流速爲 0.25 mL min^{-1}，樣品注射量 5 μL。葉黃素、α-及 β-胡蘿蔔素標準品 (Sigma-Aldrich Chemical Co., St Louis, MO, USA)，純度 99.6%，購自友和貿易股份有限公司。

結果與討論

類胡蘿蔔素標準品配製爲 μg mL^{-1} 之濃度，利用氰甲烷及甲醇爲移動相，以等位沖提方式可同時分析及定量葉黃素、α-及 β-胡蘿蔔素 (圖 1)。沖提出之順序及滯留時間分別爲葉黃素 2.28 min、α-胡蘿蔔素 15.87 min 及 β-胡蘿蔔素 16.85 min。每一類胡蘿蔔素標準品皆可建立良好之線性關係 (R^2 = 0.99) (圖 2)，此方法可在 17 min 內完成蔬果中，3 種結構相近之類胡蘿蔔分離及定量。於類胡蘿蔔素標準曲線之線性範圍內，進行同日間 (within-day) 及異日間 (between-day) 試驗。配製的標準溶液於不同的 6 d 重覆注射 6 次，每次注射 5 μL，將所得之數據 (retention time 及 peak area) 計算標準偏差 (standard deviation) 與變異係數 (coefficient of variation) (表 1)。Within-day 之滯留面積變異係數 (% CV) 分別爲葉黃素 0.5、α-胡蘿蔔素 0.4 及 β-胡蘿蔔素 0.74。Between-day 之滯留面積變異係數分別爲葉黃

表 1. 葉黃素、α-胡蘿蔔素及 β-胡蘿蔔素滯留時間及波峰面積之再現性。

Table 1. The reproducibility of retention time and peak area of lutein, α-carotene and β- carotene.

	Within-day (n = 6)				Between-day (n = 6)			
	Retention time		Peak area		Retention time		Peak area	
Item	X ± SD[z]	% CV[y]	X ± SD	% CV	X ± SD	% CV	X ± SD	% CV
Lutein	2.28 ± 0.005	0.02	8344.5 ± 12.6	0.50	2.29 ± 0.01	0.31	7887.5 ± 69.1	0.88
α-carotene	15.87 ± 0.004	0.05	8527.3 ± 32.6	0.40	15.86 ± 0.18	1.11	8550.4 ± 31.3	0.37
β-carotene	16.85 ± 0.007	0.03	3147.8 ± 23.2	0.74	16.84 ± 0.18	1.08	3103.8 ± 59.9	1.90

[z] SD: standard deviation.
[y] CV: coefficient of variation.

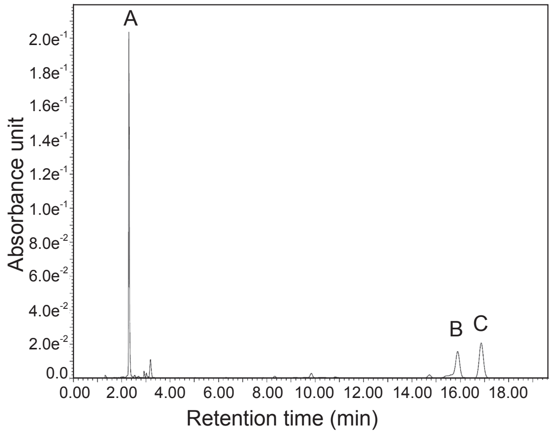

圖 1. (A) 葉黃素、(B) α-胡蘿蔔素及 (C) β-胡蘿蔔素標準品之 UPLC (Ultra Performance Liquid Chromatography) 圖譜。

Fig. 1. The UPLC (Ultra Performance Liquid Chromatography) profile of authentic lutein (A), α-carotene (B), and β-carotene (C).

素 0.88、α-胡蘿蔔素 0.37 及 β-胡蘿蔔素 1.9，顯示其精確度 (precision) 良好。於市售蔬菜樣本中加入含葉黃素 0.3 μg、α-胡蘿蔔素 16.8 μg 及 β-胡蘿蔔素 0.32 μg 之對照標準品，以測定其回收率。測得平均回收率為 102.8、105.4 及 101.7%。

本研究成功利用 UPLC 精準及快速分離定量蔬菜的三種類胡蘿蔔素。由於類胡蘿蔔素具生理活性，對人體健康有益，因此過去有許多研究報告，探討以 HPLC (high-performance liquid chromatography) 儀器建立類胡蘿蔔素分析技術與方法。相較於傳統 HPLC，UPLC 儀器的改良使分析微量化合物上可較精準且快速。目前利用 HPLC 同時分析多種類胡蘿蔔素的資料中，多數方法分析時間需耗費 30–60 min，速度不及 UPLC，過程也需耗費大量有機溶劑 (Barua 2001; Aman *et al*. 2005; Dias *et al*. 2010; Gleize *et al*. 2012; Montesano *et al*. 2012)。相較於其他利用 UPLC 分析葉黃素之方法，本法使用溶劑簡單，若單以分析葉黃素為標的，分析時間可於 5 min 內完成 (Hung *et al*. 2011; Liu *et al*. 2011; Li *et al*. 2012)。由於整個分析過程只需少量有機溶劑甲醇及氰甲烷 (共 5 mL)，所以分析成本較傳統使用之 HPLC

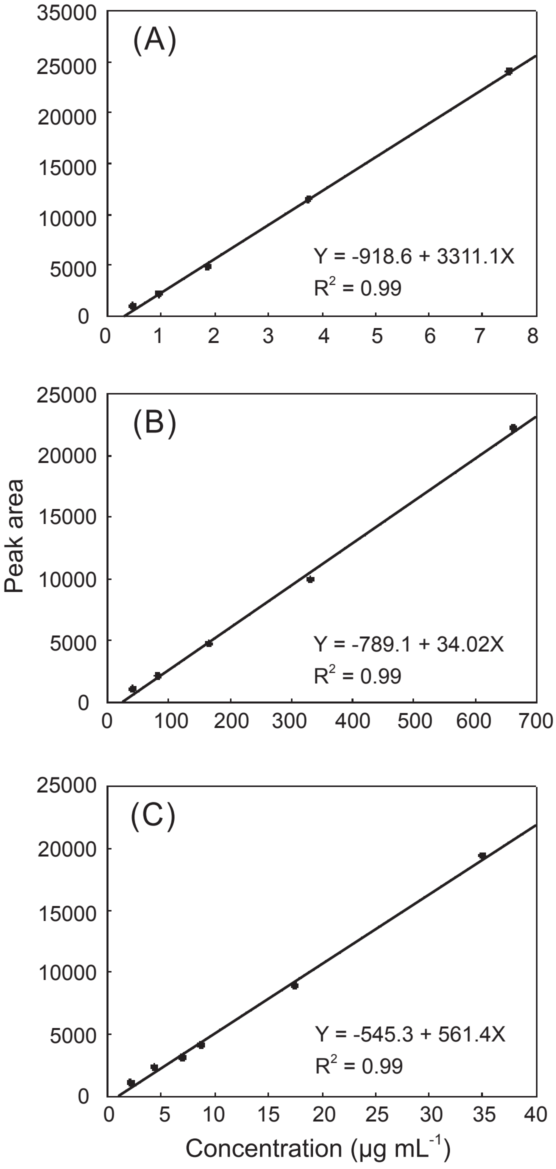

圖 2. 類胡蘿蔔素之 (A) 葉黃素、(B) α-胡蘿蔔素及 (C) β-胡蘿蔔素標準品檢量線。

Fig. 2. Linearity of response of authentic lutein (A), α-carotene (B), and β-carotene (C).

為低，產生的廢液又少，對環境友善。若分析標的需延伸至胡蘿蔔素，亦可增加移動相速度進一步縮短整個分析時間。在葉黃素波峰出現後，伴隨 3.19 min 處有一小波峰 (圖 1)，依文獻推測可能為玉米黃素 (zeaxanthin) 或其它 lutein isomer，至於結構為何需進一步鑑定

(Aruna & Baskaran 2010; Liu *et al*. 2011; Fu *et al*. 2012)。

以上述之葉黃素分析條件，進行國內常食用之 24 種蔬菜中葉黃素含量調查，並於分析過程中添加內部標準品 β-apo-8’-carotenal 以校正葉黃素的測定值 (表 2)。表中未註明莖或葉者，則以全株計算。結果顯示葉黃素含量介於 0.16–1.77 mg g^{-1} 乾重。所有檢測樣本中以香菜葉葉黃素含量最高，達 1.77 mg g^{-1} 乾重。葉黃素含量大過 1 mg g^{-1} 乾重者有共有 8 件樣本，除了香菜葉外，其他如香菜全株 (1.42 mg g^{-1} 乾重)、川七 (1.28 mg g^{-1} 乾重)、九層塔 (1.2 mg g^{-1} 乾重)、小茴香 (1.17 mg g^{-1} 乾重)、芥藍菜葉 (1.25 mg g^{-1} 乾重) 及本島萵苣 (1.05 mg g^{-1} 乾重)，外觀多屬深綠色之綠葉蔬菜。其他高葉黃素含量之蔬菜，介於 0.69–0.87 mg^{-1} 乾重，如茼蒿 (0.8 mg g^{-1} 乾重)、空心菜 (0.69 mg g^{-1} 乾重)、油菜 (0.6 mg g^{-1} 乾重) 及菠菜 (0.87 mg g^{-1} 乾重) 等。而許多綠葉蔬菜之葉部葉黃素又較全株或莖部含量爲高，如空心菜葉 (0.83 mg g^{-1} 乾重)、油菜葉 (0.84 mg g^{-1} 乾重) 等。綠葉蔬菜類葉黃素含量多數高於黃色蔬菜及淡色蔬菜，但淡色蔬菜之外葉不食用部位，如花椰菜等，而葉片呈現越深綠者，葉黃素含量越高，皆大過食用部位，相差約 0.32 mg g^{-1} 乾重。瓜果類蔬菜如大黃瓜，其未食用之綠色外皮含量亦高過於白色果肉組織，約 0.53 mg g^{-1} 乾重，兩者相差達 2.6 倍，可見許多未食用之農業廢棄蔬菜，葉黃素含量明顯高過食用部位。黃色蔬菜如南瓜，葉黃素含量也不低 (0.52 mg g^{-1} 乾重)，或許因本試驗選擇綠皮種的南瓜所致。

由於科技進步，使用液晶螢幕機會增加，人體對葉黃素的需求提升，因此許多研究報告特別針對作物葉黃素含量進行探討，包括蔬果 (Murillo *et al*. 2010; Kao *et al*. 2012)、小麥 (Lachman *et al*. 2013)、油料作物 (Habib *et al*. 2013) 及香料植物 (Aruna & Baskaran 2010)。可見許多國家研究當地作物的葉黃素含量，希望能增加自己國民攝取量以及健康。台灣蔬菜葉黃素含量，分析結果顯示與Kao *et al*. (2012) 相同，以香菜葉黃素含量最高，香辛類之配菜九層塔含量亦高於其他蔬菜。

以本研究結果對照國外學者所收集的分析樣本，不論作物類別爲何，葉黃素仍以深綠色葉之作物含量較高 (Ishiguro & Yoshimoto 2005; Aruna & Baskaran 2010; Chandrika *et al*. 2010; Murillo *et al*. 2010)。本研究中葉黃素含量與文獻資料不完全相符，可能爲蔬菜中的類胡蘿蔔素含量會因爲蔬菜品種、部位、採收時間、種植條件及環境氣候造成差異 (Ishiguro & Yoshimoto 2005)。各類蔬菜經化學分析結果配合外觀顏色之差異，大致可歸納出葉黃素含量高低不同之蔬菜組群。消費者在過度使用眼睛的情形下，可大約由蔬菜外觀色澤差異，選得葉黃素含量較高且喜愛或習慣性食用的蔬菜食用，藉以補充體內所需葉黃素，以達身體保健目的 (Burgos *et al*. 2009)。

文獻指出每天攝取 6–14 mg 的葉黃素，能有效降低 50% 的 AMD 發生機率 (Alves-Rodrigues & Shao 2004)。以本研究結果計算成人葉黃素建議攝取量，以較不受颱風影響的空心菜爲例，所測得葉黃素含量爲 0.57 mg g^{-1} 乾重，以一般蔬菜水含量 95% 計算，表示每天攝食空心菜 200 g 即可達到葉黃素的建議攝取量。若食用芥藍菜等葉黃素含量更高之蔬菜，只需約 100 g 即可。地瓜葉也是台灣的盛產蔬菜，依學者研究結果顯示其葉黃素含量爲 31.5–42.6 mg 100 g^{-1} 鮮重 (Ishiguro & Yoshimoto 2005; Kao *et al*. 2012)，亦顯示每日每人只需攝食約 100 g，即可達葉黃素建議攝取量。依文獻指出許多水果亦含高量的葉黃素，藉以攝取水果補充葉黃素，亦爲一好方法 (Perry *et al*. 2009; Murillo *et al*. 2010)。

由結果顯示，許多未食用之農業廢棄蔬菜，如大黃瓜皮或蔬菜外葉，葉黃素含量高過食用部位，在糧食短缺及葉黃素對人體重要性日漸提升之現況下，此些廢棄蔬菜未來應可進一步作多元化之加值利用。另外，目前許多研究致力於生質能源之發展，未來若能配合生質能源之製程將葉黃素萃取出純化，以作爲醫學保健產品之原料等多項應用，亦爲一良好的量產葉黃素模式。

本研究以甲醇及氰甲烷爲移動相之等位沖

表 **2.** 國人常食用之蔬菜葉黃素含量表。

Table 2. Lutein content (mg g^{-1} dry weight) of vegetables commonly consumed in Taiwan.

Vegetable name	Scientific name	mg g^{-1} D. W.	Remarks
Coriander	*Coriandrum sativum* L.	1.42 ± 0.21	
Coriander leaf	*C. sativum* L.	1.77 ± 0.20	
Coriander stem	*C. sativum* L.	0.61 ± 0.03	
Chinese knot weed	*Polygonum chinensis* L.	1.28 ± 0.11	
Basil	*Ocimum basilicum* L.	1.20 ± 0.03	
Common dill	*Anethum graveolens* L.	1.17 ± 0.11	
Black mustard	*Brassica nigra* (L.) W. D. J. Koch	1.10 ± 0.07	
Black mustard leaf	*B. nigra* (L.) W. D. J. Koch	1.25 ± 0.10	
Black mustard stem	*B. nigra* (L.) W. D. J. Koch	0.60 ± 0.11	
Lettuce	*Lactuca sativa* L.	1.05 ± 0.09	
Garland chrysanthemum	*Chrysanthemum coronarium* L.	0.80 ± 0.02	
Prickly-seeded spinach	*Spinacia oleracea* L.	0.78 ± 0.06	
Prickly-seeded spinach leaf	*S. oleracea* L.	0.87 ± 0.01	
Prickly-seeded spinach stem	*S. oleracea* L.	0.57 ± 0.02	
Edible rape	*Brassica campestris* L.	0.60 ± 0.05	
Edible rape leaf	*B. campestris* L.	0.84 ± 0.10	
Edible rape stem	*B. campestris* L.	0.48 ± 0.08	
Asparagus lettuce	*Lactuca sativa var. asparagina* Bailey	0.78 ± 0.01	
Chinese leek	*Allium tuberosum* Rottl. ex K. Spreng.	0.71 ± 0.12	
Pumpkin	*Cucurbita maxima* Duchesne ex Lam.	0.52 ± 0.16	
Bell pepper	*Capsicum annuum* var. *annuum* Grossum group	0.44 ± 0.08	
Mustard	*Brassica juncea* (L.) Czerniak. et Coss.	0.65 ± 0.05	
Mustard leaf	*B. juncea* (L.) Czerniak. et Coss.	0.77 ± 0.04	
Mustard stem	*B. juncea* (L.) Czerniak. et Coss.	0.37 ± 0.01	
Water convolvulus	*Ipomoea aquatica* Forssk.	0.69 ± 0.05	
Water convolvulus leaf	*I. aquatica* Forssk.	0.83 ± 0.04	
Water convolvulus stem	*I. aquatica* Forssk.	0.57 ± 0.01	
Bayam selaseh	*Amaranthus caudatus* L.	0.60 ± 0.03	
Bayam selaseh leaf	*A. caudatus* L.	0.94 ± 0.03	
Bayam selaseh stem	*A. caudatus* L.	0.43 ± 0.05	
Welsh onion	*Allium fistulosum* L.	0.53 ± 0.02	
Welsh onion leaf	*A. fistulosum* L.	0.69 ± 0.02	
Pak-choi	*Brassica campestris* L. ssp. Chinensis (L.) Mak.	0.47 ± 0.12	
Pak-choi leaf	*B. campestris* L. ssp. Chinensis (L.) Mak.	0.55 ± 0.10	
Pak-choi stem	*B. campestris* L. ssp. Chinensis (L.) Mak.	0.39 ± 0.03	
Turnip pulp	*Brassica campestris* L. ssp. rapifera Metzg.	0.47 ± 0.06	
Turnip peel	*B.campestris* L. ssp. *rapifera* Metzg.	0.59 ± 0.01	Inedible
Turnip leaf	*B.campestris* L. ssp. *rapifera* Metzg.	0.75 ± 0.05	Inedible
Cauliflower	*Brassica oleracea* L. Botrytis Group	0.56 ± 0.01	
Cauliflower outer leaf	*B. oleracea* L. Botrytis Group	0.88 ± 0.11	Inedible

表 2. 國人常食用之蔬菜葉黃素含量表 (續)。

Table 2. Lutein content (mg g^{-1} dry weight) of vegetables commonly consumed in Taiwan. (continued)

Vegetable name	Scientific name	mg g^{-1} D. W.	Remarks
Cabbage	*Brassica oleracea* L.	0.49 ± 0.05	
Cabbage outer leaf	*B. oleracea* L.	0.54 ± 0.03	Inedible
Cucumber	*Cucumis sativus* L.	0.32 ± 0.07	
Cucumber peel	*C. sativus* L.	0.85 ± 0.09	Inedible
Pai-tsai	*Brassica campestris* L. ssp. *pekinensis* (Lour.) Olsson	0.42 ± 0.05	
Pai-tsai leaf	*B. campestris* L. ssp. *pekinensis* (Lour.) Olsson	0.52 ± 0.03	
Pai-tsai outer leaf	*B. campestris* L. ssp. *pekinensis* (Lour.) Olsson	0.63 ± 0.08	Inedible
Amaranth	*Amaranthus mangostanus* L.	0.24 ± 0.01	
Amaranth leaf	*A. mangostanus* L.	0.27 ± 0.07	
Amaranth stem	*A. mangostanus* L.	0.16 ± 0.08	

提方式可同時分析及定量葉黃素、α-及 β-胡蘿蔔素等 3 種類胡蘿蔔素化合物。所建立的台灣常食用的蔬菜之葉黃素含量資料庫，可作為國人選擇健康飲食的參考依據。

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An Improved Ultra Performance Liquid Chromatography Method for Analyzing Lutein Content of Vegetables in Taiwan

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Abstract

Lo, S. C. and P. E. Chung. 2014. An improved ultra performance liquid chromatography method for analyzing lutein content of vegetables in Taiwan. J. Taiwan Agric. Res. 63(2):159–166.

Lutein can mitigate eyes' aging and macular degeneration, but cannot be synthesized in the human body. It must be supplemented from diet. An efficient method for separation and quantification of lutein was developed to establish the database of lutein contents of various commercial vegetables in Taiwan. By ultra performance liquid chromatography, lutein can be analyzed within 5 min using ethylene bridged hybrid (BEH) C18 as a column and an isocratic elution solution of acetonitrile/methanol (70 : 30, v/v) at a flow rate 0.25 mL min^{-1}. The α-carotene and β-carotene could also be determined simultaneously. The results showed that lutein contents ranged between 0.16 to 1.77 mg g^{-1} dry weights among 50 vegetable samples. The lutein contents of eight samples were higher than 1 mg g^{-1} dry weight, and most of them were dark green leafy vegetables, including coriander, water spinach, rapeseed and Chinese kale. The highest was coriander's leaves, up to 1.77 mg g^{-1} dry weight. The contents of lutein in the dark green leafy parts were higher than the stems of the yellow and white vegetables. However, the contents of lutein in leaves of the inedible parts of white vegetables were higher than the edible parts. The lutein content of cucumber peel was higher than the pulp up to 2.6 times. According to the differences between vegetable colors and lutein contents, consumers can choose dark green leafy vegetables to increase daily intake of lutein. The content of lutein in the inedible parts of vegetables, usually regarded as agricultural wastes, is higher than in the edible parts. Therefore, these wastes are valuable for value-adding applications in the future.

Key words: Lutein, Vegetables, Ultra performance liquid chromatography.

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鳳梨「台農 21 號 (黃金)」之育成

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摘要

唐佳惠、蔡孟勳、蔡惠文、官青杉。2014。鳳梨「台農 21 號 (黃金)」之育成。台灣農業研究 63(2):167–177。

鳳梨「台農 21 號 (黃金)」是由行政院農業委員會農業試驗所嘉義農業試驗分所，自母本 C64-4-117 (‘Smooth Cayenne’ ×「台農 4 號」) 與父本 C64-2-56 (‘Smooth Cayenne’ × ‘Rough’) 之雜交後裔，歷經 20 餘年選育而成。經 2005 年 6 月通過農委會果樹品種審議委員會審議，命名為「台農 21 號」，商品名為「黃金鳳梨」。本品種平均果重 1.34 kg，果實圓筒形，果肉黃或金黃色，肉質緻密，平均可溶性固形物 18.9 ºBrix，無裂梗、果心斷裂及裂目 (裂果) 及肉聲果比例偏高等生理問題。果實採收適期為 4–11 月，貯藏性佳。

關鍵詞：「台農 21 號」、黃金鳳梨、育種。

前言

鳳梨 [*Ananas comosus* (L.) Merrill] 爲我國重要經濟果樹之一，據農業統計年報 (2012 年版) 資料顯示，目前的栽培面積爲 9,335 ha，產量 392,221 Mg，產值 72.2 億元，主要產區分佈於屏東縣、台南市、高雄市、嘉義縣及南投縣等地。國內早期的鳳梨產業係以栽種「開英種」 (Hsu 1952) 發展罐頭加工爲主，故曾爲國際上重要的鳳梨罐頭出口國之一。隨著國際產業結構更替，台灣鳳梨加工產業逐漸外移而沒落，爲謀產業轉型，各界無不致力於將以加工爲導向的產業調整成發展鮮食市場爲主軸。經過多年努力由國內試驗改良場所陸續推出可獲國內、外鮮食鳳梨消費市場青睞的品種 (Chang 1991)，並快速而順利的輔導農民成功轉型。台灣在鮮食鳳梨選育工作之進展十分迅速，行政院農業委員會農業試驗所嘉義農業試驗分所自 1975 年起重新投入雜交選種的工作，自 1994 年起即命名推廣「台農 13 號 (冬蜜)」。近二十年來更陸續推廣「台農 16 號 (甜蜜蜜)」、「台農 17 號 (金鑽)」、「台農 18 號 (金桂花)」及「台農 19 號 (蜜寶)」等 4 個品種，加上農業試驗所鳳山分所於 2004 年命名推廣的「台農 20 號 (牛奶)」，平均 3 年餘即有一個新品種的推出。這些品種各有喜愛其品質特性的消費者，然目前栽培面積最多者仍爲「台農 17 號」。

「台農 17 號」目前占總栽培面積之 70% 以上，不但是國內市場的主力品種，亦爲外銷量最多者。該品種具備豐產、栽培管理容易及鮮食品質佳等優點，深受產銷雙方之肯定。惟此品種在環境不理想時易有裂梗、裂目 (裂果) 現象，且在南部栽培時夏果品質易有劣化等缺點 (Tang & Kuan 2012)，尚待其他品種補足此一缺失。有鑑於此，嘉義農業試驗分所以具備不易裂梗、不易果心斷裂或裂目 (裂果) 及肉聲果比例低者爲目標，自選種圃中選拔有潛力解決上述「台農 17 號」栽培問題的品種，期能提供鳳梨農友另一項選擇。本文報告「台農 21

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號」之選育過程，試驗比較其與「開英種」及「台農 17 號」之性狀差異，並歸納其生產優缺點，期供未來利用及推廣之參考。

材料與方法

鳳梨「台農 21 號」選拔時之品系號碼為 C75-2-25，係 1986 年以品系號碼 C64-4-117 (♀ 'Smooth Cayenne' × ♂「台農 4 號」) 為母本，與品系號碼 C64-2-56 (♀ 'Smooth Cayenne' × ♂ 'Rough') 為父本，進行雜交選育而得之優良後代。取得雜交種子後，經 3 年的實生苗培育工作，於 1990 年開始初選，獲優良後裔計 18 株系，共採集種苗 50 株。複選工作陸續於 1990–1992 年期，1992–1994 年期，1994–1996 年期，1996–1998 年期及 1998–2000 年期等 5 期 (代) 進行，共獲得 6 個優良株系。C75-2-25 品系於 1999 年起，在嘉義試驗分所選種果園中進行品系比較試驗，2002–2005 年在屏東、高雄、台南及嘉義等地進行區域試驗。

雜交實生苗培育與初選

1986 年期共雜交 9 個組合，計 130 株 (果) 10,884 朵花，收穫種子 12,450 粒，於同年 10 月份將收穫之種子播種培育，半年後苗高約 3 cm 時移植至溫室內苗床繼續培養，待苗高約 15 cm 時移植於網室苗床，最後培育至苗高約 30 cm 時移至果園定植。每次移植時先將葉緣有刺之種苗淘汰。果園規劃係按不同親本組合，分別依苗之大小順序排列。畦、行及株距分別為 100 cm × 50 cm × 30 cm，採 2 列式三角形種植。肥培管理按一般鳳梨之栽培方法進行 (生育期間視植株生長所需，妥善進行施肥及病蟲害防治作業) ，栽植 1 年後進行植株生育調查，果實成熟時調查果重及果實特性，並以手持屈折計 (Atago PAL-1 Co. Ltd, Tokyo, Japan) 測定可溶性固形物含量。

初選優良株系繁殖與複選

培育選拔而得之實生苗，於 1990 年初選獲得 18 優良品系，共採集種苗 50 株，同年 11 月種植供複選之用。果園規劃方式及肥培管理等作業與初選時相同，供試植株栽植 1 年後進行生長調查，果實成熟採收時調查果實特性及品質，供作複選之依據。每期作獲選株系繼續採集後裔供繁殖與複選，並進行老株塊莖切片繁殖，以加速繁殖種苗擴大族群，供品系比較試驗。複選工作連續於 1990–1992 年期，1992–1994 年期，1994–1996 年期，1996–1998 年期及 1998–2000 年期等五期 (代) 進行。

品系比較試驗

比較試驗於 1999 年 11 月設區種植，由 C65-7-187、C69-8-105、C71-8-142、C71-6-153 及「台農 21 號」等 5 品種 (系) 參試，對照品種為「台農 17 號」，共 6 品種 (系)，4 重複，計 24 小區。每小區面積為 3 m × 4.5 m (13.5 m^2)，種植 60 株，全試區種植 1,440 株，採逢機完全區集設計。試區栽植後按一般栽培方法進行肥培管理，定期調查植株生長情形、抽穗期、成熟期、果重、果實特性及吸芽數等。採收時每小區隨機取 20 顆果實樣品，以屈折計測定可溶性固形物含量，含酸量以 0.1 N 的 NaOH 標準液，滴定至 pH 8.1 之體積，換算為檸檬酸之百分比 (%)。

品系區域試驗

「台農 21 號」區域試驗分別於 2002–2004 年期及 2003–2005 年期兩期作進行，以「台農 17 號」與「開英種」為對照，3 品種，5 重複，計 15 小區，採逢機完全區集設計。試驗果園之施肥依 N-P-K 為 16 : 4 : 16 之三要素比例，每株施 76 g 硫酸銨、22 g 過磷酸鈣及 32 g 硫酸鉀，分 4 次施入土壤中。果實成熟時每小區隨機採 20 顆果實調查平均果重，並依比較試驗所述之方法，測定可溶性固形物及可滴定酸含量。2002–2004 年期之試驗地點計有嘉義縣民雄鄉、台南市大內區、高雄市大樹區及屏東縣高樹鄉；2003–2005 年期計有嘉義縣民雄鄉、屏東縣瑪家鄉及高雄市內門區共 7 處。

2002–2004 年期各試驗區均於 2002 年秋植，並於栽植後滿 1 年進行生育調查，各試區任其自然開花結果，以生產 2004 年 6–7 月之夏果。2003–2005 年期，各試驗區均於 2003

年 10 月栽植，翌年 12 月進行生育調查，各試驗區於10月初利用0.1%電石水 (碳化鈣加水，產生乙炔氣體) 催花，2005年3月底開始採收。

鳳梨萎凋病調查

2002–2004 年期在 4 處區域試驗果園，同時期陸續調查參試品種，即「台農21號」、「台農 17 號」及「開英種」的萎凋病發生情形。

貯藏性試驗

爲瞭解鳳梨「台農 21 號」果實的貯藏性，本試驗以重量 1.6 kg 左右的果實，自果園採收後模擬集運至集貨場時調查果實擦壓傷情形，之後於 25℃、13℃及 10℃下進行貯藏。並以同一農戶所生產，果重相類似的「台農 17 號」鳳梨爲對照組。貯藏試驗爲期 2 週，貯藏後每週取出一次供品質分析。

結果

初選優良株系繁殖與複選

1986 年期雜交後代初選獲得 6 個優良品系，其性狀詳如表 1。其中 C75-2-25 (即「台農 21 號」) 平均株高 97.8 cm，葉長 93.4 cm，葉寬 6.2 cm，葉數 41 片，葉綠色，平均果重 1.66 kg，略微圓筒形。成熟果皮色黃較厚，花腔淺。果肉黃或金黃色，纖維及肉質細緻。可溶性固形物 17 ºBrix (表 1)，鮮食風味佳。

品系種比較試驗

以 C65-7-187、C69-8-105、C71-8-142、C71-6-153、「台農 21 號」及「台農 17 號」進行的比較試驗，在果實成熟採收後秤取其重量，並於每小區隨機取樣 20 顆果實供品質分析，顯示：5 個供試品種 (系) 中，果實重量以 C65-7-187、C71-8-142 與「台農 17 號」三者較低，分別爲 1,273 g、1,324 g 與 1,396 g，其次是 C71-6-153 與「台農 21 號」，分別爲 1,508 g 及 1,527 g，而 C69-8-105 最高，達 1,587 g。「台農 21 號」果肉顏色鮮黃至金黃色，肉質緻密，鳳梨特有風味濃郁，鮮食品質佳。且估算的每公頃產量可較對照品種 (「台農 17 號」) 增產 9%，其可溶性固形物達 18.8 ºBrix，可滴定酸含量 0.55%，固酸比爲 34.7，略高於「台農 17 號」之 32.2 (表 2)。顯示，「台農 21 號」在果實重量、可溶性固形物及可滴定酸含量高，與「台農 17 號」二者間，差異均達 5% 統計上顯著水準。

品系區域試驗

2002–2004 年期各試區之果實於 2004 年 6–7 月採收。「台農 21 號」平均果重在各試驗區介於 1,073–1,490 g 之間，公頃產量介於 38,600–53,700 kg。較之「台農 17 號」的平均果重介於 1,080–1,622 g，公頃產量 38,800–58,400 kg，而「開英種」平均果重 1,268–1,693 g，公頃產量 45,600–61,000 kg，均較

表 1. 1986 年期鳳梨雜交後代初選優良品系性狀 (夏果)[z]。

Table 1. The characteristics of selected pineapple[z] hybrids in primary trial (summer fruit).

Selection	PH[y] (cm)	LL (cm)	LW (cm)	NL	FW (g)	FL (cm)	FrW (cm)	NS	FTS	PC	FCD (cm)	PF	PT	TSS (ºBrix)
C75-2-25	97.8	93.4	6.2	41	1,660	15.5	12.5	216	Pong	Golden	2.5	Smooth	+++[x]	17.0
C75-3-205	104.2	106.8	6.6	32	1,640	17.5	12.2	150	Pier	Golden	3.5	Rough	+++	10.2
C75-7-16	119.0	96.5	5.2	36	1,840	15.9	13.6	120	Pong	Light yellow	2.8	Smooth	++	14.2
C75-7-81	87.6	68.8	5.4	39	1,720	18.4	12.7	117	Pong	Yellow	3.7	Smooth	++	16.4
C75-8-31	116.2	97.8	6.0	42	1,900	20.9	12.2	228	Pier	White	4.8	Rough	++	9.6
C75-8-79	87.6	94.6	6.2	38	1,620	15.2	12.6	204	Pong	Golden	2.5	Rough	++	12.4

[z] Data were mean of 20–30 fruits.

[y] Abbreviation, PH: Plant height; LL: Leaf length; LW: Leaf Width; NL: Number of leaf; FW: Fruit weight; FL: Fruit length; FrW: Fruit width; NS: Number of the scales; FTS: Fruit tapping sound; PC: Pulp color; FCD: Fruit core diameter; PF: Pulp fiber; PT: Pulp texture; and TSS: Total soluble solids.

[x] +: loose; ++: medium; +++: dense; ++++: bally dense.

低，且差異達 5% 顯著水準 (表 3)。台南市大內區試驗區因植株發育稍差，3 個供試品系 (種) 平均果重均顯著較其他試區輕。在 4 個試驗區中，「台農 21 號」僅嘉義縣民雄鄉試驗區之平均果重略大於「開英種」而顯著小於「台農 17 號」及屏東縣高樹鄉試區顯著較「台農 17 號」重；餘均小於「開英種」而與「台農 17 號」無顯著差異 (表 3)。「台農 21 號」在各試驗區之固酸比介於 23.2–35.3 之間，其中可溶性固形物含量介於 18.0–19.7 ºBrix 之間，均顯著高於二個對照品種；可滴定酸介於 0.54–0.82% 之間，除嘉義縣民雄鄉及高雄市大樹區兩試驗區外，均顯著較「開英種」低，而與「台農 17 號」無顯著差異 (表 3)。

表 2. 1993 年夏果品系比較試驗各品系 (種) 平均果重、可溶性固形物及可滴定酸含量比較。

Table 2. Comparisons of weight per fruit, total soluble solids and titratable acidity contents among ‘TN17’ and five other selected lines in 1993.

		40,000 pl ha^{-1}						
Variety/line	Weight per fruit (g)	Yield (Mg ha^{-1})	Index (%)	TSS[z] (ºBrix)	TA (%)	TSS/TA ratio	Pulp fiber	Pulp texture
C65-7-187	1,273 c[y]	50.9	91	16.4 b	0.39 c	42.5 a	Rough	+++[x]
C69-8-105	1,587 a	63.6	114	15.9 c	0.42 b	37.5 b	Smooth	++
C71-6-153	1,508 b	60.3	108	16.6 b	0.64 a	27.9 c	Smooth	++
C71-8-142	1,324 c	53.0	95	15.8 c	0.45 b	36.9 b	Smooth	++
TN21 (C75-2-25)	1,527 b	61.1	109	18.8 a	0.55 a	34.7 b	Medium	+++
TN17 (CK)	1,396 c	55.8	100	14.5 d	0.45 b	32.2 b	Medium	+++

[z] Abbreviation, TSS: Total soluble solids; TA: Titratable acidity.
[y] Means followed by the same letters at the same column within the same year were not significantly different at 5% level by LSD test. LSD: least significant difference.
[x] +: loose; ++: medium; +++: dense; ++++: bally dense.

表 3. 2002–2004 年期「台農 21 號」區域試驗之平均果重、公頃產量、可溶性固形物及可滴定酸含量綜合比較[z]。

Table 3. Comparisons of weight per fruit, yield, total soluble solids and titratable acidity contents among pineapple ‘TN21’ (C75-2-25), ‘TN17’ and ‘Smooth Cayenne’ in 2002–2004[z].

City/township	Variety	Weight per fruit (g)	Yield (Mg ha^{-1})	Index of yield (%)	TSS[y] (ºBrix)	TA (%)	TSS/TA ratio
Chiayi/ Minsyong	TN21	1,490 b[x]	53.7	105	18.6 a	0.82 a	23.2 a
	TN17	1,622 a	58.4	114	13.5 b	0.66 b	22.3 a
	Smooth Cayenne	1,421 b	51.2	100	14.4 b	0.77 a	19.1 b
Tainan/ Danei	TN21	1,073 b	38.6	85	18.0 a	0.54 b	35.3 a
	TN17	1,080 b	38.8	85	12.3 c	0.59 b	21.7 b
	Smooth Cayenne	1,268 a	45.6	100	14.8 b	0.74 a	20.7 c
Kaohsiung/ Dashu	TN21	1,476 b	53.2	89	19.1 a	0.57 a	34.2 a
	TN17	1,511 b	54.4	91	15.8 b	0.55 a	29.4 b
	Smooth Cayenne	1,658 a	59.7	100	14.8 c	0.53 b	30.1 b
Pingtung/ Gaoshu	TN21	1,341 b	48.3	79	19.7 a	0.69 b	29.8 a
	TN17	1,221 c	44.0	72	16.3 b	0.65 b	25.9 b
	Smooth Cayenne	1,693 a	61.0	100	14.8 c	0.74 a	20.9 c
Mean	TN21	1,345	48.5	90	18.9	0.66	30.6
	TN17	1,359	48.7	91	14.5	0.61	24.8
	Smooth Cayenne	1,510	54.4	100	14.7	0.70	22.7

[z] The seedlings for autumn planting in 2002 and the fruits were harvested in June and July of 2004.
[y] Abbreviation, TSS: Total soluble solids; TA: Titratable acidity.
[x] Means followed by the same letters at the same column within the same year were not significantly different at 5% level by LSD test. LSD: least significant difference.

2003–2005 年期各試驗區之果實於 2005 年3月底採收。「台農21號」平均果重1,228 g，公頃產量 44,200 kg，與 2 對照品種「台農 17 號」之 1,274 g，公頃產量 45,900 kg 及「開英種」之 1,566 g，公頃產量 56,400 kg 相較，分別減產 3.7% 及 21.6%，且差異均達 5% 顯著水準。該年度各試驗區植株發育受 2004 年前半年乾旱之影響，平均產量均低於 2002–2004 年期者，但仍與該年期呈現「台農 21 號」小於「開英種」而與「台農 17 號」類似的趨勢 (表 4)。「台農 21 號」之可溶性固形物在 3 個試驗區均顯著高於 2 個對照品種，且差異達 5% 統計上顯著水準；而可滴定酸含量除屏東縣瑪家鄉試驗區與 2 個對照品種無顯著差異外，餘均顯著高於「台農 17 號」而與「開英種」無顯著差異，固酸比亦顯著高於「開英種」(表 4) 。

鳳梨萎凋病調查

2002–2004 年期在 4 處區域試驗果園，調查3個供試品種 (系) 的萎凋病發生情形，顯示：罹病率受各試驗區果園環境與栽培管理差異而影響。整體而言台南市大內區試驗區最嚴重，各品種 (系) 罹病率均高於一成，以「台農 17 號」最高，為 19.13%，「開英種」17.3% 次之；而「台農 21 號」罹病率稍低，為 14%，與二個對照品種差異達 5% 統計上之顯著水準。屏東縣高樹鄉及嘉義縣民雄鄉試驗區有零星發生，而高雄市大樹區試驗區則無 (表 5)。

貯藏性試驗

「台農 21 號」與「台農 17 號」鳳梨貯藏於 10℃、13℃和 15℃下，對果實品質之影響如表 6 所示。在相對較低的貯藏溫度下，「台農 21 號」的失重率、可溶性固形物及可滴定酸含量之變化趨勢與「台農 17 號」類似；但在常溫下貯藏 2 週後，「台農 21 號」的失重率似較「台農 17 號」高。在果皮外觀異常方面，於各試驗溫度下貯藏 1 週的「台農 17 號」果皮外觀異常之情形皆較「台農 21 號」嚴重。於 13℃及 10℃下貯藏 2 週後，「台農 21 號」鳳梨果實外觀亦明顯較「台農 17 號」佳。

討論

Hume and Miller (1904) 指出，供商業生產之鳳梨可分為開英系 (Cayenne)、西班牙系 (Spanish) 及奎恩系 (Queen)。開英系果肉呈淡黃色，小果較大，植株生育強，葉片稍直立，是世界上栽培較廣的加工與鮮食兼用種 (Wang

表4. 2003–2005年期「台農21號」區域試驗之平均果重、公頃產量、可溶性固形物及可滴定酸含量綜合比較[z]。

Table 4. Comparisons of weight per fruit, yield, total soluble solids and titratable acidity contents among pineapple 'TN21' (C75-2-25), 'TN17' and 'Smooth Cayenne' in 2003–2005[z].

City/township	Variety	Weight per fruit (g)	Yield (Mg ha^{-1})	Index of yield (%)	TSS[y] (ºBrix)	TA (%)	TSS/TA ratio
Chiayi/ Minsyong	TN21	1,309 c[x]	47.1	76	16.7 a	0.58 a	29.2 a
	TN17	1,526 b	54.9	89	13.8 c	0.50 b	27.6 a
	Smooth Cayenne	1,715 a	61.7	100	15.4 b	0.63 a	24.7 b
Kaohsiung/ Neimen	TN21	1,124 b	40.5	71	19.3 a	0.64 a	30.8 a
	TN17	1,157 b	41.7	73	16.7 b	0.59 b	28.7 a
	Smooth Cayenne	1,579 a	56.8	100	15.5 b	0.69 a	22.8 b
Pingtung/ Majia	TN21	1,251 b	45.1	89	17.5 a	0.55 ab	32.2 a
	TN17	1,139 c	41.0	81	15.4 b	0.51 b	30.9 a
	Smooth Cayenne	1,404 a	50.6	100	15.4 b	0.61 a	25.2 b
Mean	TN21	1,228	44.2	78	17.8	0.59	30.7
	TN17	1,274	45.9	81	15.3	0.53	29.1
	Smooth Cayenne	1,566	56.4	100	15.4	0.64	24.2

[z] The seedlings were planted in October 2003 and forced-flowering in October 2004.The fruits were harvested in April and May of 2005.

[y] Abbreviation, TSS: Total soluble solids; TA: Titratable acidity.

[x] Means followed by the same letters at the same column within the same year were not significantly different at 5% level by LSD test. LSD: least significant difference.

表 5. 鳳梨果園萎凋病罹病率調查。

Table 5. Investigation of wilt disease rate in pineapple orchards.

City/township	Variety	Number of plant	Number of diseased plant	Morbidity (%)
Chiayi/Minsyong	TN21	300	2	0.66 a[z]
	TN17	300	0	0.00 a
	Smooth Cayenne	300	0	0.00 a
Tainan/Danei	TN21	300	42	14.00 b
	TN17	300	58	19.33 a
	Smooth Cayenne	300	52	17.33 a
Kaohsiung/Dashu	TN21	300	0	0.00 a
	TN17	300	0	0.00 a
	Smooth Cayenne	300	0	0.00 a
Pingtung/Gaoshu	TN21	300	0	0.00 a
	TN17	300	12	4.00a
	Smooth Cayenne	300	2	0.66a

[z] Means followed by the same letters at the same column within the same year were not significantly different at 5% level by LSD test. LSD: least significant difference.

表 6. 「台農 21 號」與「台農 17 號」鳳梨貯藏於 10、13 和 25℃下 2 週後於室溫下櫥架 4 d 對果實品質之影響。

Table 6. Effect of temperature on the quality of ‘TN21’ and ‘TN17’ pineapple fruit, which are stored two weeks and then returned to ambient air at 25℃ for 4 days.

Storage temperature (℃)	Variety	Fruit weight loss (%)	Abnormal symptom of peel	Fruit translucency	IB[z]	TSS (°Brix)	TA (%)
10	TN21	6.18 c[y]	0.6 c	0.4 b	1.2 b	16.0 a	0.67 a
	TN17	6.35 c	2.9 a	2.4 a	4.4 a	13.6 b	0.54 b
13	TN21	7.56 b	1.3 b	1.1 b	0.8 b	16.4 a	0.63 ab
	TN17	7.72 b	2.9 a	2.4 a	4.2 a	13.5 b	0.58 ab
25	TN21	11.16 a	2.5 a	0.4 b	0.0 c	16.5 a	0.39 c
	TN17	7.56 b	1.6 b	0.9 b	0.0 c	12.5 b	0.54 b

[z] IB: Internal browning (the symptom of chilling injury); TSS: Total soluble solids; TA: Titratable acidity.

[y] Means followed by the same letters at the same column within the same year were not significantly different at 5% level by LSD test. LSD: least significant difference.

& Chang 1957; Lin 1978)；西班牙系果肉呈白色，小果扁平，葉片強硬有刺，但萎凋病的抗性較強 (Bartolome *et al*. 1995)；奎恩系果肉為黃色，果汁多，植株生育中等 (Chan & Lee 1995)。由過去育種經驗顯示，育種時為選拔果園罹病率低、不易生理劣變及肉聲果比例低之性狀，但又符合目前鮮食市場品質需求之品種，其親本需跨越不同系統之間。由於「台農 17 號」為「開英種」(smooth cayenne) 與 ‘Rough’ 的雜交種，具有植株中等、產量高、果形整齊美觀等特點，栽培得法時果實品質較一致，品質上較無肉聲果及鼓聲果之別，兼具葉緣無刺等特性 (Chang *et al*. 1999)，因此在鮮食品質上要能與「台農 17 號」相擬比者，可能需在親本上有一定程度的相似。「台農 21 號」與「台農 17 號」，皆屬開英系與奎恩系的雜交種，其在葉刺的著生位置及果實外觀 (表 1；Kuan *et al*. 2006) 的表現上具有推廣潛力；在產量的表現上，綜合各區域試驗結果顯示，「台農 21 號」公頃產量約 48,500 kg，雖較「開英種」的 54,400 kg 低，然與「台農 17 號」的 48,700 kg 類似 (表 3)，並高於目前國內每公頃平均產量 47,878 kg (2012 年版農業統計年報)，顯示本品系的產量亦可符合目前栽培者對品種特性之需求。

栽培者對鳳梨品種的需求，除葉刺及產量之外，管理技術的難易也是重要的影響因子。例如「台農 17 號」的建議產期為每年 3–6 月，

然在目前栽培比率高達 70% 以上的情形下，農民爲避免盛產期爆量供貨而影響收益，常將產期往前提早至 1–2 月，或往後延遲使果實成熟期落於每年 7 月至翌年 2 月。由於近年全球暖化、氣候變遷與異常氣候經常發生，致溫差及乾濕變化加劇，尤其 2 月及 10 月時的氣候情況常爲溫度仍高、潮濕有雨，造成該時段抽穗之植株易發生裂梗或裂果等問題 (Tang & Kuan 2012)，必需增加管理技術之層次。而栽種於南部之「台農 17 號」若要生產夏果，則需能克服夏季品質劣化的困擾。裂梗問題似不存在於「台農 21 號」，在 2002–2004 及 2003–2005 兩個期別的區域試驗過程，即使是生產正常產期的夏果 (抽穗期在 2 月中)，均未見到裂梗情形。而延遲催花以提早在 3 月開始採收果實 (抽穗期在前一年的 11 月中)，亦未見到裂梗情形。由此顯示，改種「台農 21 號」確實可避免栽培「台農 17 號」時需克服的裂梗問題。

另一個影響栽培者選種品種的因素，乃肉聲果產出比率。由於鳳梨果實可利用軟棒敲彈果實，聽取其發出的聲音來輔助判斷果實品質之好壞，通常依其反射音大致可分爲肉聲果、鼓聲果及杜聲果 (Kuan & Hsu 1997; Sornsrivichai *et al*. 2000)，其口感、甜酸度及風味，甚至貯放時間均各不相同。市面上以肉聲果和鼓聲果較多 (Chang 1993)，一般鼓聲果的纖維較細、甜度高，風味較濃郁，但可滴定酸含量亦高。而肉聲果含水量多、肉質較粗且不耐放 (Chen 2001; Huang & Cho 2009)。僅管如此，春季若選購「開英種」鼓聲果，易因可滴定酸含量較高顯得食用品質太差；若選購肉聲果則可因可滴定酸含量稍減而提高適口性，然此情形在夏果又未必適用。加上其他的鮮食品種如「台農 18 號」及「台農 19 號」等，因肉聲果比率稍高，且杜聲果及鼓聲果的鮮食品質常較肉聲果佳 (Kuan & Hsu, 1997; Cheng, 2002)，因此栽培者對鳳梨果實反射音呈現肉聲果之品種普遍存在疑慮。由於「台農 21 號」無肉聲果比例偏高的生理問題，多數均爲鼓聲果且肉聲果或鼓聲果品質差異不明顯，因此改種「台農 21 號」亦可減輕肉聲果造成之困擾。

栽培者喜歡的鳳梨品種，除了要上述的生理問題少、植株較矮、葉緣無刺、果型整齊而大、品質優良並耐旱等，也會考慮其抗 (耐) 病能力。鳳梨萎凋病是常見的病害之一，由粉介殼蟲媒介病毒傳播，故以防治媒介昆蟲爲主 (Tao 1950; Lin & Tsai 2001; Su 2001)，選種耐、抗病品種或不帶病之種苗爲輔。台灣南部在乾季期間 (10 月至翌年 5 月)，罹病植株自外圍老葉開始變色，逐漸向內層葉片進展，葉尖開始乾枯、失去膨脹性並下垂，最後僅剩中心數葉爲綠色。罹病株有生育緩慢、植株矮小、不能抽穗結果、果實不能正常發育、果實萎縮、果目突出及果肉纖維化等現象，致無食用價值 (Lin 1997)。目前國內命名的鮮食品種中，僅「台農 19 號 (蜜寶)」較耐萎凋病，果園發病率極低。「台農 21 號」在 2002–2004 年期發育狀況較差的台南市大內區試驗區，果園罹病率爲 14%，但較兩對照品種低 (表 5)，顯示「台農 21 號」在果園管理不當或發育較差的情形下，果園萎凋病罹病率仍然較對照品種少；而在良好栽培管理下，則不易出現萎凋病 (表 5)。

除了栽培層面的技術問題之外，食用品質爲影響消費面的重點之一，本品種選育目標之一即爲克服夏季不易購得品質優良鳳梨之困擾。由於在食用品質方面，「台農 21 號」夏果 (表 2) 及春果 (表 4) 的可溶性固形物均顯著高於「台農 17 號」，而兩品種之可滴定酸含量在夏果 (表 2 及表 3) 及春果 (表 4) 的表現上，則受到栽培地區之影響。栽種於嘉義之試驗果園者，其差異均達 5% 統計上之顯著水準，而栽種於南部產區者則無顯著差異。由此顯示，「台農 21 號」無「台農 17 號」夏果甜味變淡的問題。進一步與「開英種」相比，則不論春果或夏果，「台農 21 號」可溶性固形物均較高而可滴定酸含量均較低，且差異均達 5% 統計上之顯著水準，可知本品種夏果之風味與口感比「開英種」優異。

目前國內鳳梨的栽培面積爲 9,335 ha，年產量達 392,000 Mg，盛產期集中於 4–8 月間，然栽培品種過度集中於「台農 17 號」，雖此情形對於發展外銷屬有利因素；然若外銷量未能持續推展，則需以品種多樣化增加選擇來提

昇消費量，否則一旦過產則易導致市場量多而滯銷。由於鳳梨屬於組織含水量多、易因搬運而擦壓傷之園產品，除加強貯藏技術研發之外，選種耐貯藏的品種亦是方法之一。「台農 17 號」果實耐貯藏性佳，可供外銷之用 (Tang *et al*. 2005)，而「台農 21 號」較「台農 17 號」的貯藏性更佳，在 13℃及 10℃下貯藏後，再經 4 d 的模擬櫥架，對照品種「台農 17 號」果肉已出現明顯的褐化寒害症狀，而「台農 21 號」果實則較輕微 (圖 1)。顯示，「台農 21 號」亦具備外銷潛力。

結論

綜合各試區的試驗結果及品種性狀之檢定 (表 7)，顯示「台農 21 號 (黃金)」具有如下特性：(1) 葉緣無刺，葉色淡綠，果梗短 (圖 2A)，植株發育旺盛 (圖 2B)。(2) 果實圓筒形，成熟果皮黃帶綠色 (圖 2C)，果皮略厚花腔淺，肉色金黃，質緻密細緻 (圖 2D)。(3) 栽培期間無裂梗、果心斷裂及裂目，肉聲果或鼓聲果實之品質差異小。(4) 平均單果重 1,340 g，可溶性固形物 18.9 ºBrix，可滴定酸含量 0.66%，貯藏性佳。(5) 果實採收適期為 4–11 月。

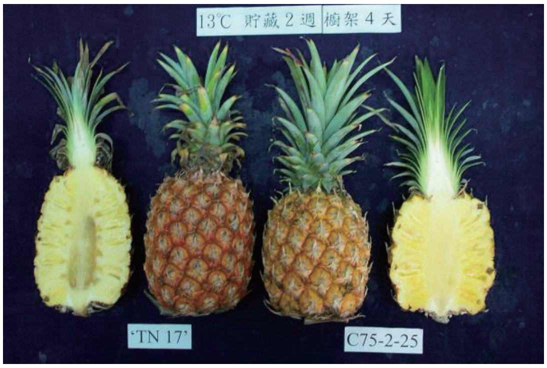

圖 1. 13℃貯藏 2 週取出置於 25℃櫥架 4 d 後之果實及果肉外觀。

Fig. 1. The appearance of peel and pulp of pineapple fruit stored for two weeks at 13℃ and then returned to ambient air at 25℃ for 4 days.

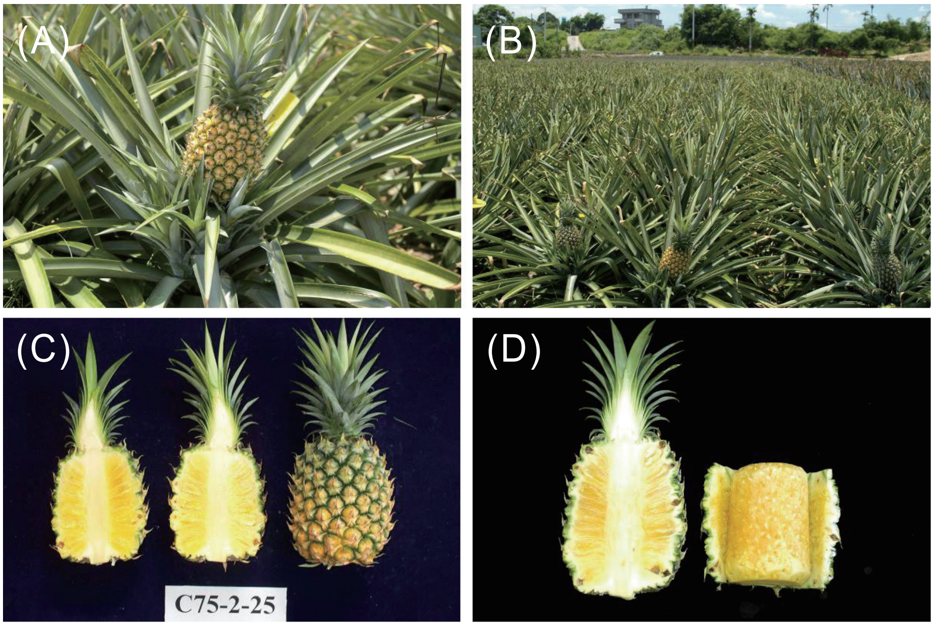

圖 2. 「台農 21 號」(C75-2-25) 之植株外觀及果實特性。(A) 葉緣無刺，葉色淡綠，果梗短；(B) 發育旺盛；(C) 果實圓筒形，成熟果皮黃帶綠；(D) 果皮略厚，花腔淺，肉色金黃，質緻密細緻。

Fig. 2. The plant appearance and characteristics of fruit of pineapple 'TN21' (C75-2-25). (A) Leaf margin was stingless, leaf color was light green and fruit pedicle was short. (B) The growth vigor was strong. (C) The fruit shape was cylindrical and the peel color of mature fruit was yellow in green. (D) The peel was slightly thick, the locule was shallow, the pulp color was golden and the pulp texture was dense.

表 7. 「台農 21 號」與對照品種「台農 17 號」及「開英種」氣候期與植物性狀比較 (調查於農業試驗所嘉義農業試驗分所)。

Table 7. Phenology and growth characteristics of 'Tainung No. 21 Golden' (C75-2-25) and two other examined comparable varieties at Chiayi Agricultural Experiment Station of Taiwan Agricultural Research Institute.

Plant part	Growth characteristics	TN21	TN17	Smooth Cayenne
Plant	Plant height	Bally short	Short	high
	Growth vigor	Strong	Strong	Strong
Leaf	Leaf length	Short	Short	Long
	Leaf width	Medium	Medium	Medium
	Leaf thickness	Medium	Medium	Medium
	Length/width ratio	Medium	Small	Slender
	Leaf luster	Light	Light	Light
	Leaf texture	Medium	Medium	Medium
	Leaf thorn	Apical without margin	Basal and apical	Basal and apical
	Leaf color	Green	Green with purple-red	Green with light purple-red
	Blade pubescence	symmetrical	symmetrical	symmetrical
Flower	Petal color	Dark purple	Light purple	Purple
	Self-incompatibility	Yes	Yes	Yes
Bud	Crown weight	Heavy	Medium	Heavy
	Crown length	Long	Medium	Long
	Number of slip	Few	Few	Medium
	Number of aerial sucker	Few	Few	Few
	Number of ground sucker	Medium	Few	Few
Fruit	Weight of per fruit	Medium	Medium	Bally heavy
	Fruit length	Medium	Medium	Long
	Medium of fruit diam.	Large	Medium	Large
	Fruit shape	Cylindrical	Cylindrical	Cylindrical
	Number of the scales	Medium	Many	Medium
	Size of the scales	Medium	Medium	Large
	Thickness of the scales	A little concave	Flat	Flat
	Fruit core diam.	Medium	Large	Medium
	Ovary interspace	Medium	Medium	Medium
	Locule	Shallow	Shallow	Medium
	Peel color	Yellow	Orange yellow with purple-red	Yellow
Other	Pulp texture	Medium	Medium	Medium
	Pulp firmness	Soft	Medium	Medium
	Pulp color	Golden	Yellow	Light yellow
	Pulp content	High	High	High
	Total soluble solids	High	Low	Medium
	Titratable acidity	High	Low	Medium
	Stem-end splitting	Non	High	Low
	Cracking peel	Non	High	High

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A New Variety of Pineapple [*Ananas comosus* (L.) Merr.] 'TN21 Golden'

Chia-Hui Tang[1], Meng-Hsun Tsai[2], Hui-Wen Tsai[1], and Ching-San Kuan[3,*]

Abstract

Tang, C. H., M. H. Tsai, H. W. Tsai, and C. S. Kuan. 2014. A new variety of pineapple [Ananas comosus (L.) Merr.] 'TN21 Golden'. J. Taiwan Agric. Res. 63(2):167–177.

A new pineapple variety 'Tainung No.21 Golden' has been selected and released by the Chiayi Agricultural Experiment Branch, Taiwan Agricultural Research Institute in June 2005. The 'Tainung No.21 Gold' pineapple fruits were in medium size (1.34 kg on average), cylindrical shape, with a yellow peel at edible ripe stage. The pulp was soft, close texture, juicy, yellow to intense yellow, with high sugar content (18.9 ºBrix on average) and intermediate to high acidity. This cultivar was not occurred stem-end splitting, pineapple core rupture and cracking peel. Fruits were harvested from April to November yearly.

Key words: 'TN21', Golden pineapple, Breeding.

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